10th

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

SEARCH REQUEST FORM

8-235

Requestor's Name: ~~ZISKA~~ Serial Number: 919097

Date: ~~8/16/93~~ Phone: ~~X1017~~ Art Unit: 1804

Search Topic:

Please write a detailed statement of search topic. Describe specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples or relevant citations, authors keywords, etc., if known. For sequences, please attach a copy of the sequence. You may include a copy of the broadest and/or most relevant claim(s).

(1) ~~plants~~ ~~yeast~~ homologous recombination using YACs (yeast artificial chromosomes)

(2) transgenic mice produced using YACs

2-bases
Biosis CAS Dialog
Medline

STAFF USE ONLY

Date completed:
Searcher: ~~Sheppard~~
Terminal time:
Elapsed time:
CPU time:
Total time:
Number of Searches:
Number of Databases:

Search Site
- STIC
- CM-1
- Pre-S

Type of Search
- N.A. Sequence
- A.A. Sequence
- Structure
- Bibliographic

Vendors
- IG Suite
- STN
- Dialog
- APS
- Geninfo
- SDC
- DARC/Questel
- Other

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?b411
      18aug93 10:29:11 User219784 Session A6.2
File 411:DIALINDEX(tm)

DIALINDEX(tm)
   (Copr. DIALOG Info.Ser.Inc.)

*** DIALINDEX search results display in an abbreviated ***
*** format unless you enter the SET DETAIL ON command. ***
?sf allsci
   You have 163 files in your file list.
   (To see banners, use SHOW FILES command)
?s (yac? ? or yeast(w)artificial(w)chromosom?)(s)(transgen?)
 Your SELECT statement is:
   s (yac? ? or yeast(w)artificial(w)chromosom?)(s)(transgen?)
          Items   File
          -----   ----
              8     5: BIOSIS PREVIEWS_69-93/JUL BA9606:BARRM4505
              1     6: NTIS_1964-1993/Sep B2
              2    10: AGRICOLA_1979-1993/Aug
              4    16: PTS PROMT_ - 72-93/August 18
              5    50: CAB Abstracts_1984-1993/Jul
              6    60: CRIS/USDA_1993/Aug
             10    73: EMBASE (EXCERPTA MEDICA)_74-93/ISS33
              5    76: Life Sciences Collection_1978-1993/Jun
              1   103: ENERGY SCIENCE & TECHNOLOGY_1974-9307B2
      Examined  50 files
              3   144: PASCAL_1973-1993/Aug
              1   148: TRADE AND INDUSTRY INDEX_81-93/AUG W3
              8   155: MEDLINE_1966-1993/OCT (9310W2)
              1   156: TOXLINE_ 1965-1993/JUL
              5   159: Cancerlit_1963-1993/Aug
             15   265: Federal Research in Progress_1993/Jul
             15   266: Federal Research in Progress_1993/Jul
      Examined 100 files
              1   285: BIOBUSINESS(R)_1985-1993/Aug W3
              6   286: BIOCOMMERCE ABS & DIRECTORY_81-93/Aug. 02
              1   315: ChemEng & Biotec Abs_1970-1993/Aug
              1   319: CHEM BUS NEWSBASE_1984-93 ISS31 /UD=9331
              1   345: INPADOC/Family & Legal Status_
              1   351: DERWENT WORLD PATENTS INDEX-LATEST_
             14   357: Derwent Biotechnology Abs._1982-1993/Aug
              1   358: CURRENT BIOTECHNOLOGY ABS_1983-1993/SEP
              7   399: CA SEARCH_1967-1993 UD=11906
             10   434: SCISEARCH(R)_1974 - 9307W4
             16   440: CURRENT CONTENTS SEARCH(R)_1990-1993/Aug W1
              1   545: INVESTEXT_82-17 Aug. 93
              3   624: McGraw-Hill Publications Online_85193/AUG 16
      Examined 150 files
              4   636: PTS NEWSLETTER DATABASE_87-93/Aug. 18

   30 files have one or more items; file list includes 163 files.

?s (yac? ? or yeast(w)artificial(w)chromosom?)(s)(transgen?)(s)(recombin?)
 Your SELECT statement is:
   s (yac? ? or
yeast(w)artificial(w)chromosom?)(s)(transgen?)(s)(recombin?)
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          Items   File
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              1      6: NTIS_1964-1993/Sep B2
              2     60: CRIS/USDA_1993/Aug
              1     73: EMBASE (EXCERPTA MEDICA)_74-93/ISS33
              1     76: Life Sciences Collection_1978-1993/Jun
      Examined  50 files
              1    144: PASCAL_1973-1993/Aug
              1    155: MEDLINE_1966-1993/OCT (9310W2)
              2    265: Federal Research in Progress_1993/Jul
              2    266: Federal Research in Progress_1993/Jul
      Examined 100 files
              3    357: Derwent Biotechnology Abs._1982-1993/Aug
              1    434: SCISEARCH(R)_1974 - 9307W4
              1    440: CURRENT CONTENTS SEARCH(R)_1990-1993/Aug W1
      Examined 150 files

   11 files have one or more items; file list includes 163 files.

?b 5 6 60 73 76 144 155 265 266 351 357 434
      18aug93 10:43:41 User219784 Session A6.3

SYSTEM:OS  - DIALOG OneSearch
  File   5:BIOSIS PREVIEWS  69-93/JUL BA9606:BARRM4505
         (c) 1993 BIOSIS
 **FILE  5: Biosystematic Codes (BC=) for viruses have changed for 1993.
 Type ?NEWS5 for more information and a complete list of the new codes.
  File   6:NTIS  1964-1993/Sep B2
         (c) 1993 NTIS
 **FILE006: See HELP NTISCODE for current prices for NTIS documents.
 Call NTIS at 703/487-4650 to order.
  File  60:CRIS/USDA  1993/Aug
  File  73:EMBASE (EXCERPTA MEDICA)  74-93/ISS33
         (c) 1993 ESP BV
 **FILE073: Truncate EMTREE Codes(e.g. DC=C1.120?) for complete retrieval.
 The 1993 Embase Thesaurus is now available.
  File  76:Life Sciences Collection  1978-1993/Jun
         (c) 1993 Cambridge Sci Abs
  File 144:PASCAL  1973-1993/Aug
         (c) 1993 INIST/CNRS
 **FILE144: Limit problem; see HELP NEWS144.
  File 155:MEDLINE  1966-1993/OCT (9310W2)
  File 265:Federal Research in Progress  1993/Jul
 **FILE265: Research project summaries from EPA (SF=EPA) and Bureau of
            Mines (SF=INTBM) are now available in FEDRIP.
  File 266:Federal Research in Progress  1993/Jul
 **FILE266: Research project summaries from EPA (SF=EPA) and Bureau of
            Mines (SF=INTBM) are now available in FEDRIP.
  File 351:DERWENT WORLD PATENTS INDEX-LATEST
         1981+;DW=9325,UA=9318,UM=9246
 **FILE351: Attention Derwent subscribers: Markush DARC on DIALOG is
    available.  Begin WPILM to access.
  File 357:Derwent Biotechnology Abs.  1982-1993/Aug
         (c) 1993 Derwent Pub. ltd.
  File 434:SCISEARCH(R)  1974 - 9307W4
         (c) 1993 ISI Inc.
 **FILE434: Contains complete, merged SciSearch file
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**Includes abstracts as of 1991

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      Set  Items  Description
      ---  -----  -----------
?s (yac? ? or yeast(w)artificial(w)chromosom?)
Processed  10 of  12 files ...
Completed processing all files
           6171  YAC? ?
         249973  YEAST
         311154  ARTIFICIAL
         563963  CHROMOSOM?
           3792  YEAST(W)ARTIFICIAL(W)CHROMOSOM?
      S1    8296  (YAC? ? OR YEAST(W)ARTIFICIAL(W)CHROMOSOM?)
?s s1 and transgen?
           8296  S1
          52373  TRANSGEN?
      S2     209  S1 AND TRANSGEN?
?s s2 (s)recombin?
            209  S2
         406296  RECOMBIN?
      S3      39  S2 (S)RECOMBIN?
?rd
>>>Duplicate detection is not supported for File 60.
>>>Duplicate detection is not supported for File 351.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S4      28  RD (unique items)
?t s4/7/1-28
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4/7/1 (Item 1 from file: 6)

1681492 NTIS Accession Number: PB93-864684/XAB

Yeast Artificial Chromosomes (YAC). (Latest citations from the Life Sciences Collection Database)

(Published Search)

NERAC, Inc., Tolland, CT.

Corp. Source Codes: 103588000

Sponsor: National Technical Information Service, Springfield, VA.

Apr 93 183 citations minimum

Languages: English Document Type: Bibliography

Journal Announcement: GRAI9313

Prepared in cooperation with Cambridge Scientific Abstracts, Washington, DC. Sponsored in part by National Technical Information Service, Springfield, VA.

NTIS Prices: PC N01/MF N01

Country of Publication: United States

The bibliography contains citations concerning the development and application of yeast artificial chromosomes. Citations include YAC applications in gene mapping, development of transgenic mice, construction of gene libraries, teleomere and centromere analysis, chromosome walking, mitotic recombination studies, and analysis of genetic diseases. Applications of YAC with drosophila, human, tomato, mouse, bacteria, and caenorhabditis elegans cells are described. In vivo and in vitro studies are also referenced. (Contains a minimum of 183 citations and includes a subject term index and title list.).

4/7/2 (Item 1 from file: 60)

09160259
PROJ NO: TEX08225 AGENCY : CRGO TEX
PROJ TYPE: CRGO
START: 15 SEP 92 TERM: 30 SEP 95
INVEST: GIOVANNONI J J
HORTICULTURAL SCIENCE
TEXAS A&M UNIV
COLLEGE STATION TEXAS 77843

HIGH DENSITY MAPPING AND ISOLATION OF GENES REGULATING TOMATO FRUIT RIPENING

OBJECTIVES:PROJ. #9201614. RFLP mapping of the rin, nor and Nr tomato fruit ripening loci. Physical mapping of the rin locus. Chromosome walking in available tomato *YAC* library. Development of ripening gene transient expression assay for localization of targeted genes within *recombinant* *YAC* inserts. Identification of the rin locus within *recombinant* *YAC* clones.

APPROACH:Employ available RFLP markers and tightly linked molecular markers isolated via RAPD analysis for mapping in segregating populations. Hybridization of genetically linked molecular markers with high molecular weight genomic DNA separated by pulsed field gel electrophoresis. Hybridization of available tomato YAC library with linked markers, followed by IPCR amplification of insert termini and re-screening if necessary. Utilization of DNA/micro projectile bombardment technology. Phenotypic complementation in transgenic plants.

PROGRESS:9201 TO 9212
Fruit ripening represents a system of development control unique to plant species. As such, analysis of the genetic components of the ripening process is likely to lead to both elucidation of the molecular mechanism(s) which control it and manipulation of specific ripening parameters via biotechnology. As indicated in item 84 above, this project (and laboratory) was initiated within the last three months. Considerable effort has been directed toward establishment of a modern and functional molecular biology laboratory which is now operational and being used for initial experiments directed toward isolation and characterization of genes involved in the ripening process. A genetic approach, whose first step is based on reliable determination of fruit ripening phenotype in segregating populations, has been selected for isolation of three ripening regulatory genes (rin, nor, Nr.). In collaboration with Monsanto Co. we have recently developed a reliable seedling screen, based on ethylene insensitivity, for the Nr mutant. This screen will allow us to conduct segregation analysis, on single lab bench, equivalent to what has previously required acres of field space. We are also in the process of physically mapping both the rin and nor loci as the second step toward cloning these genes. Markers flanking the rin and nor have been tentatively localized to 800 kb and 1000 kb restriction fragments, respectively, with current efforts directed toward reproduction of these observations.

PUBLICATIONS: 9201 TO 9212
GIOVANNONI, J. (1993) Molecular biology of fruit development and ripening. In Methods in Plant Molecular Biology. (Bryant, J. ed.) Academic Press. In press.

4/7/3 (Item 2 from file: 60)

09159562
PROJ NO: CA-D*-VCR-5571-CG AGENCY : CRGO CALB
PROJ TYPE: CRGO
START: 01 AUG 92 TERM: 31 JUL 95
INVEST: MICHELMORE R W
VEGETABLE CROPS
UNIV OF CALIFORNIA
DAVIS CALIFORNIA 95616

MAP BASED CLONING OF DISEASE RESISTANCE GENES

OBJECTIVES:PROJ. #9201723. To clone and characterize genes for resistance in Lactuca sativa to the fungus, Bremia lactucae, by map-based cloning.

APPROACH: The strategy involves the integration of classical and molecular genetics and analyzes of mutant and *transgenic* plants. PCR-based techniques will be used to dissect specific complex regions of the genome containing resistance genes. The interaction between L. sativa and B. lactucae is a powerful model system for such studies because of the ease of manipulation in the laboratory and the extensive genetic characterization of this disease. Three clusters of resistance genes will be saturated with markers. The relationship between genetic and physical distance will be determined by the analysis of *recombinants* in the region using pulsed field gel electrophoresis. The physically closest markers will be used to initiate walks to the gene using overlapping genomic clones from *YAC* or cosmid libraries. The identity of the resistance genes will be confirmed by complementation.

4/7/4 (Item 3 from file: 60)
09157082
PROJ NO: TEX08161 AGENCY : CRGO TEX
PROJ TYPE: CRGO
START: 01 SEP 91 TERM: 31 AUG 94 FY: 1992
INVEST: WING R A
SOIL & CROP SCIENCES
TEXAS A&M UNIV
COLLEGE STATION TEXAS 77843

DEVELOPMENT OF MAP-BASED CLONING IN CROP PLANTS: TOMATO AS A MODEL SYSTEM

OBJECTIVES:PROJ. #9104194. The main goal for the three year period covered by this research proposal is to initiate and establish a new area of research, based on the strengths of tomato genetics and the ability to clone genes from plants by chromosome walking, to investigate abscission zone cell differerentiation. Immediate approaches will focus on the isolation and analysis of two genes that completely suppress the pedicle abscission zone of tomato: the jointless mutants -- j-1 and j-2.

APPROACH: The specific objectives of this project are: genetically link molecular markers(RFLP, RAPD, etc.) to both the jointless genes; use the most closely linked markers as starting points to begin to "walk" to the jointless genes in a tomato yeast artificial chromosome library: continue walking until YAC clones are found to be 100% linked to each of the jointless mutations; experiments will be initiated to test positive YACs for complementation of the jointless mutants by Agrobacterium mediated transformation.

PROGRESS:9201 TO 9212
Jointless (j-1) was mapped to a 3cM interval with RFLP and RAPD markers. A *YAC* library was screened and 6 *YACs* were isolated and the *YAC* ends were mapped. *YAC* TY142 was shown to contain a contiguous piece of DNA that appears to contain the jointless gene. We are now taking several approaches to locate the jointless gene within TY142. 1). An additional 1000 F2 plants are being screened for *recombinants*. This experiment will allow us to more precisely position the jointless gene in the TY142. 2). We are in the process of screening two floral meristematic cDNA libraries, one tobacco with radioactively labeled TY142. In addition we are constructing our own cDNA library using RNA from various stages of abscission zone development to be screened as above. If we can isolate a candidate cDNA(s) by these experiments it will reduce the number of *transgenic* tomato plants that will be needed to prove we have cloned the jointless gene. 3). Simultaneously we are subcloning TY142 into the plant transformation vectors SLJ1711 or SLJ44024A for tomato transformation. 4). NIL materials for both j and j-2 have been obtained for complementation experiments. Experiments are under way to evaluate the best NIL to use with respect to transformation frequency. Jointless-2 (j-2). Mapping j-2 in a interspecific cross between L. esculentum and L. pennellii proved difficult because the jointless phenotype was hard to score. However, preliminary data suggests that j-2 is not on chromosome 11.

PUBLICATIONS: 9201 TO 9212
PATERSON, A. and WING, R.A. 1993. Genome mapping in plants. Current Opinion in Biotechnology 4: (###-###). TANKSLEY, S.D., GANAL, M.W., PRINCE, J.C., de VICENTE, M.C., BONIERBALE, M.W., BROUN, P. FULTON, T.M., GIOVANNONI, J.J., GRANDILLO, S. MARTIN, G.B., MESSEQUER,R., MILLER, J.C., MILLER, L., PATERSON, A.H., PINEDA, O., ROEDER, M.S., WING, R.WING, R.A., *GIOVANNONI, J.J., GANAL, M. and TANKSLEY, S.D. 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acid Research 23:6553-6558.(* Co-First Authors). WING, R.A., ZHANG, H.B. and TANKSLEY, S. Molecular mapping of jointless. In preparation for M.G.G. WING, R.A., RASTOGI, V., ZHANG, H., PATERSON, A. and TANKSLEY, S. Embedding plant protoplasts in agarose microbeads for the purposes of physical mapping andYAC cloning. LAN, T.H., PATERSON, A.H. and WING, R.A. Integration of two RFLP maps using a common mapping population. Submitted to Tomato Genetics Cooperative Newsletter.

4/7/5 (Item 1 from file: 73)
8826315 EMBASE No: 93129086
Rapid physical mapping of YAC inserts by random integration of I-Sce I sites
Colleaux L.; Rougeulle C.; Avner P.; Dujon B.
Unite Genetique Moleculaire/Levures, Departement de Biologie Moleculaire, Institut Pasteur, 25 Rue du Dr Roux, F-75724 Paris Cedex 15 France
HUM. MOL. GENET. (United Kingdom) , 1993, 2/3 (265-271) CODEN: HMGEE ISSN: 0964-6906
LANGUAGES: English SUMMARY LANGUAGES: English
We have developed a novel strategy, based on the random insertion by homeologous *recombination* of artificial I-Sce I sites within mammalian repetitive DNA sequences, which should greatly facilitate the high resolution physical mapping of large DNA fragments cloned in *YAC*. A set of *transgenic* yeast strains containing appropriately spaced I-Sce I sites within the *YAC* insert defines a series of nested physical intervals against which new genes, clones or DNA fragments can be mapped by simple

hybridisation. Sequential hybridisation using such a series of nested *YAC* fragments as probes can also allow the rapid sorting of phage or cosmid libraries into contigs. This approach, which has been applied to a *YAC* containing a 460 kb insert from the mouse X chromosome, may also have applications for the restriction mapping of large genomic segments, mapping of exons and the search for homologous genes.

4/7/6 (Item 1 from file: 155)
07957944 92095944
Transfer of yeast artificial chromosomes from yeast to mammalian cells.
Huxley C; Gnirke A
Department of Genetics, Washington University Medical School, St. Louis, MO 63110.
Bioessays Oct 1991, 13 (10) p545-50, ISSN 0265-9247 Journal Code: 9YY
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
Human DNA can be cloned as *yeast* *artificial* *chromosomes* (*YACs*), each of which contains several hundred kilobases of human DNA. This DNA can be manipulated in the yeast host using homologous *recombination* and yeast selectable markers. In relatively few steps it is possible to make virtually any change in the cloned human DNA from single base pair changes to deletions and insertions. In order to study the function of the cloned DNA and the effects of the changes made in the yeast, the human DNA must be transferred back into mammalian cells. Recent experiments indicate that large genes can be transferred from the yeast host to mammalian cells in tissue culture and that the genes are transferred intact and are expressed. Using the same methods it may soon be possible to transfer *YAC* DNA into the mouse germ line so that the expression and function of genes cloned in *YACs* can be studied in developing and adult mammalian animals. (40 Refs.)

4/7/7 (Item 1 from file: 265)
0104511 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 2R01ES05194-04A1 AGENCY CODE: CRISP
Human serum paraoxonase--Role in pesticide metabolism
PRINCIPAL INVESTIGATOR: FURLONG, CLEMENT E
ADDRESS: UNIVERSITY OF WASHINGTON SEATTLE, WA 98195
PERFORMING ORG.: UNIVERSITY OF WASHINGTON, SEATTLE, WASHINGTON
SPONSORING ORG.: NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
FY : 93 FUNDS: $243,776 TYPE OF AWARD: Competing Continuation (Type 2)
SUMMARY: Paraoxonase/arylesterase is a serum enzyme that is associated with HDL particles. It hydrolyzes the toxic oxons of several organophosphorus insecticides and the nerve agents soman and sarin. This enzyme exhibits a substrate dependent polymorphism in humans. One isoform, with arginine at position 192, hydrolyzes paraoxon with a low turnover number. Other substrates such as chlorpyrifos oxon and phenylacetate are hydrolyzed by both isoforms with the same turnover number. The first major objective is to determine the role of human serum paraoxonase in the metabolism of toxic organophosphorus compounds. Purified (natural or recombinant) high and low activity isoforms will be injected into mice and the in vivo protection afforded by each isoform will be determined. Transgenic mice will be constructed which express either the human high or low activity isoform. The transgenic animals will be challenged with organophosphates and the protection provided. by. each isoform determined. The constructs for expression of paraoxonase in the transgenic mice will

make use of the cDNAs or subclones from yeast artificial chromosome (YAC) clones already characterized. Epidemiological studies will determine whether serum paraoxonase status affects the response of humans to organophosphorus compounds. Paraoxonase genotype and levels of expression will be determined in individuals with histories of toxicity and compared with values in individuals from the same population who have not experienced episodes of toxicity. PCR, immunological and enzyme assays developed in the first phase of this research will be used to establish the paraoxonase status of each individual. The second major objective is to define the role of paraoxonase in lipid metabolism. Lipase activity will be determined with a number of lipid substrates. Correlations of paraoxonase status with lipoproteins and lipid levels will be examined. The third major objective is to continue the molecular genetic studies on the structure and regulation of the paraoxonase gene. The sequencing and fine structure mapping will be completed and the 5' and 3' noncoding regions from individuals who express very high and low levels of protein win be examined for possible involvement in the regulation of the paraoxonase gene. The completion of the sequencing of the few remaining exon/intron boundries will allow amplification of individual exons from genomic DNA by the polymerase chain reaction (PCR). The amplified paraoxonse coding regions will be examined by single strand conformational polymorphism analysis in non-caucasoid ethnic groups to determine if additional variants exist.

4/7/8 (Item 2 from file: 265)
0096480 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 1R01HD28623-01A1 AGENCY CODE: CRISP
Cloning pallid, a mutant affecting inner ear and bleeding
PRINCIPAL INVESTIGATOR: ELLIOTT, ROSEMARY W
ADDRESS: HLTH RES INC, ROSWELL PARK DIV 666 ELM AND CARLTON STS BUFFALO, N Y 14263
PERFORMING ORG.: ROSWELL PARK MEMORIAL INSTITUTE, BUFFALO, NEW YORK
SPONSORING ORG.: NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
FY : 93 FUNDS: $161,071 TYPE OF AWARD: New Award (Type 1)
SUMMARY: The goal of this project is to clone a gene that complements the mouse pallid mutant. This mutant is one of a group of eleven genetically distinct mutants affecting coat color and bleeding time. These effects are mediated through alterations in melanosomes and platelet dense granules, both of which are present, but defective in the mutant mice. Some of the mutants, including pallid, also have some effects on the function of a third organelle, the lysosome. Pallid is one of three mutants that affect the inner ear, and the mutants display ataxia because the otoliths, required for balance, are missing. Cloning the normal gene will allow analysis of the normal function of the gene in all parts of its complex phenotype, both at the molecular and cellular level, and analysis of the mutant phenotype.

The proposed approach to cloning this gene involves identifying the molecular marker closest to the pallid mutation on mouse chromosome 2. This will be done using an interspecific backcross (C57BL/6J-pa a1/pa a1 X M. spretus-+ A/+ A)F1 X C57BL/6J-pa a1/pa a1, that has already produced 123 progeny. Preliminary data suggest that beta2-microglobulin is a very close marker. A YAC clone containing the selected marker will be obtained and used to start the walk to pallid. The ends of the YAC will be cloned and sequenced to identify overlapping YACs. Backcross animals containing recombination breakpoints will be identified and used first to determine the direction of the walk and later to monitor progress between the starting point and the target. YAC clones overlapping the interval between the recombination breakpoints flanking the pallid mutation will be

candidates for the desired clones.

To determine whether any of the genes on the YAC are expressed in megakaryocytes (platelet precursors) or in skin, cDNA libraries from skin and bone marrow cells will be made in a 'fragmenting vector' which contains a selectable yeast marker not present in the YAC and a yeast telomere. The libraries will be transformed into yeast cells containing the candidate YAC and recombinants between individual cDNAs and genes on the YAC will be selected. This will allow identification of one or more candidate genes, as regions between the candidate gene and the telomere will be deleted in the recombinant YACs, being replaced by vector sequences. Experiments to detect the function of the locus involve transformation of DNA from the candidate YAC or its cosmid subclones into bone marrow cells and reintroduction of these cells into mice to determine whether the fraction of platelets with filled dense granules is increased. To determine whether the pigment dilution can be corrected, transgenic mice will be made by injecting the same constructs into fertilized eggs homozygous for the mutant.

4/7/9 (Item 3 from file: 265)
0093874 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 1K11HL02711-01 AGENCY CODE: CRISP
Analysis of B globin locus regulation by YAC transfer
PRINCIPAL INVESTIGATOR: GAENSLER, KARIN L
ADDRESS: UNIVERSITY OF CALIFORNIA BOX 0444 SAN FRANCISCO, CA 94143-0444
PERFORMING ORG.: UNIVERSITY OF CALIFORNIA SAN FRANCISCO, SAN FRANCISCO, CALIFORNIA
SPONSORING ORG.: NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
FY : 93 FUNDS: $73,980 TYPE OF AWARD: New Award (Type 1)
SUMMARY: Disorders of human globin gene expression are among the most common hereditary diseases of man. Detailed analysis of specific mutations in the Beta globin gene cluster has provided insights into the mechanisms of human gene regulation, and may assist in the development of genetically-based therapies. Gene transfer studies in cell lines and transgenic mice have identified important promoter and enhancer elements of the Beta globin locus. However, it has not been possible to study the physiological role of these cos-acting sequences within the intact Beta globin gene region, due to the insert size limitations of existing plasmid and cosmid vectors. The Beta globin locus, including the 5' and 3' erythroid-specific Dnase I hypersensitive sites, spans at least 90kb, whereas cosmid vectors accommodate less than 50 kb. Our goal is to more precisely define the cis-acting sequences involved in the tissue- and stage-specific expression of the members of the Beta globin gene family. The recently developed technique for cloning genomic DNA fragments of several hundred kb or more into yeast artificial chromosomes (YACs), has now made it possible to isolate large genetic loci, such as the Beta globin gene cluster, including cis-acting sequences located in 5' and 3' flanking regions. We will combine the advantages of YAC technology with gene transfer methods, in order to introduce YACs containing the Beta globin gene locus into murine cell lines and transgenic mice. Previously, we have extensively characterized two YACs containing the entire Beta globin locus and flanking regions in a single contiguous insert. Using these YACs, we will adapt methods for reliably introducing high molecular weight DNA into cells, while preserving the genomic structure of these fragments after their integration into the host chromatin. We will assess the relative advantages of different transfection procedures using murine erythroleukemia cells or embyonic stem cells. We will also test microinjection of oocytes for this purpose. Once we have produced cell lines or mice carrying the entire human Beta globlin gene locus, we will

use quantitative RNA analysis to study the expression of the intact human Beta globin gene family. We will use homology-directed recombination in yeast to generate mutations of potential regulatory sequences in, and flanking the locus. Yacs containing Beta globin loci with specific mutations will be transferred using the procedures we have developed. The effect of these mutations on tissue- and developmental-stage specific gene expression will then be analyzed. We believe this work will provide a model system for better understanding the molecular mechanisms of gene regulation during ontogeny, as well as for studying structure/function relationships of large genetic loci, whose size has up to now precluded mutational analysis and transfer into cells.

4/7/10 (Item 4 from file: 265)
0090526 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 0160259; TEX08225 AGENCY CODE: AGRIC
HIGH DENSITY MAPPING AND ISOLATION OF GENES REGULATING TOMATO FRUIT RIPENING
PRINCIPAL INVESTIGATOR: GIOVANNONI J J
PERFORMING ORG.: TEXAS A&M UNIV, HORTICULTURAL SCIENCE, COLLEGE STATION, TEXAS 77843
SPONSORING ORG.: U. S. DEPARTMENT OF AGRICULTURE, COMPETITIVE RESEARCH GRANT OFFICE
DATES: 920813 TO 950930
SUMMARY: OBJECTIVE: PROJ. #9201614. RFLP mapping of the rin, nor and Nr tomato fruit ripening loci. Physical mapping of the rin locus. Chromosome walking in available tomato YAC library. Development of ripening gene transient expression assay for localization of targeted genes within recombinant YAC inserts. Identification of the rin locus within recombinant YAC clones.

APPROACH: Employ available RFLP markers and tightly linked molecular markers isolated via RAPD analysis for mapping in segregating populations. Hybridization of genetically linked molecular markers with high molecular weight genomic DNA separated by pulsed field gel electrophoresis. Hybridization of available tomato YAC library with linked markers, followed by IPCR amplification of insert termini and re-screening if necessary. Utilization of DNA/micro projectile bombardment technology. Phenotypic complementation in transgenic plants.

PROGRESS REPORT SUMMARY: PROGRESS: Fruit ripening represents a system of development control unique to plant species. As such, analysis of the genetic components of the ripening process is likely to lead to both elucidation of the molecular mechanism(s) which control it and manipulation of specific ripening parameters via biotechnology. As indicated in item 84 above, this project (and laboratory) was initiated within the last three months. Considerable effort has been directed toward establishment of a modern and functional molecular biology laboratory which is now operational and being used for initial experiments directed toward isolation and characterization of genes involved in the ripening process. A genetic approach, whose first step is based on reliable determination of fruit ripening phenotype in segregating populations, has been selected for isolation of three ripening regulatory genes (rin, nor, Nr.). In collaboration with Monsanto Co. we have recently developed a reliable seedling screen, based on ethylene insensitivity, for the Nr mutant. This screen will allow us to conduct segregation analysis, on single lab bench, equivalent to what has previously required acres of field space. We are also in the process of physically mapping both the rin and nor loci as the second step toward cloning these genes. Markers flanking the rin and nor have been tentatively localized to 800 kb and 1000 kb restriction

fragments, respectively, with current efforts directed toward reproduction of these observations.

4/7/11 (Item 5 from file: 265)
0090039 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 0159562; CA-D*-VCR-5571-CG AGENCY CODE: AGRIC
MAP BASED CLONING OF DISEASE RESISTANCE GENES
PRINCIPAL INVESTIGATOR: MICHELMORE R W
PERFORMING ORG.: UNIV OF CALIFORNIA, VEGETABLE CROPS, DAVIS, CALIFORNIA 95616
SPONSORING ORG.: U. S. DEPARTMENT OF AGRICULTURE, COMPETITIVE RESEARCH GRANT OFFICE
DATES: 920729 TO 950731
SUMMARY: OBJECTIVE: PROJ. #9201723. To clone and characterize genes for resistance in Lactuca sativa to the fungus, Bremia lactucae, by map-based cloning.
APPROACH: The strategy involves the integration of classical and molecular genetics and analyzes of mutant and transgenic plants. PCR-based techniques will be used to dissect specific complex regions of the genome containing resistance genes. The interaction between L. sativa and B. lactucae is a powerful model system for such studies because of the ease of manipulation in the laboratory and the extensive genetic characterization of this disease. Three clusters of resistance genes will be saturated with markers. The relationship between genetic and physical distance will be determined by the analysis of recombinants in the region using pulsed field gel electrophoresis. The physically closest markers will be used to initiate walks to the gene using overlapping genomic clones from YAC or cosmid libraries. The identity of the resistance genes will be confirmed by complementation.

4/7/12 (Item 6 from file: 265)
0088266 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 0157082; TEX08161 AGENCY CODE: AGRIC
DEVELOPMENT OF MAP-BASED CLONING IN CROP PLANTS: TOMATO AS A MODEL SYSTEM
PRINCIPAL INVESTIGATOR: WING R A
PERFORMING ORG.: TEXAS A&M UNIV, SOIL & CROP SCIENCES, COLLEGE STATION, TEXAS 77843
SPONSORING ORG.: U. S. DEPARTMENT OF AGRICULTURE, COMPETITIVE RESEARCH GRANT OFFICE
DATES: 910801 TO 940831
SUMMARY: OBJECTIVE: PROJ. #9104194. The main goal for the three year period covered by this research proposal is to initiate and establish a new area of research, based on the strengths of tomato genetics and the ability to clone genes from plants by chromosome walking, to investigate abscission zone cell differerentiation. Immediate approaches will focus on the isolation and analysis of two genes that completely suppress the pedicle abscission zone of tomato: the jointless mutants -- j-1 and j-2.
APPROACH: The specific objectives of this project are: genetically link molecular markers (RFLP, RAPD, etc.) to both the jointless genes; use the most closely linked markers as starting points to begin to "walk" to the jointless genes in a tomato yeast artificial chromosome library: continue walking until YAC clones are found to be 100% linked to each of the jointless mutations; experiments will be initiated to test positive YACs for complementation of the jointless mutants by Agrobacterium mediated transformation.
PROGRESS REPORT SUMMARY: PROGRESS: Jointless (j-1) was mapped to a 3cM interval with RFLP and RAPD markers. A YAC library was screened and 6 YACs

were isolated and the YAC ends were mapped. YAC TY142 was shown to contain a contiguous piece of DNA that appears to contain the jointless gene. We are now takingseveral approaches to locate the jointless gene within TY142. 1). An additional 1000 F2 plants are being screened for recombinants. This experiment will allow us to more precisely position the jointless gene in the TY142. 2). We are in the process of screening two floral meristematic cDNA libraries, one tobacco with radioactively labeled TY142. In addition we are constructing our own cDNA library using RNA from various stages of abscission zone development to be screened as above. If we can isolate a candidate cDNA(s) by these experiments it will reduce the number of transgenic tomato plants that will be needed to prove we have cloned the jointless gene. 3). Simultaneously we are subcloning TY142 intothe plant transformation vectors SLJ1711 or SLJ44024A for tomato transformation. 4). NIL materials for both j and j-2 have been obtained for complementation experiments. Experiments are under way to evaluate the best NIL to use with respect to transformation frequency. Jointless-2 (j-2). Mapping j-2 in a interspecific cross between L. esculentum and L. pennellii proved difficult because the jointless phenotype was hard to score. However, preliminary data suggests that j-2 is not on chromosome 11.

4/7/13 (Item 1 from file: 357)
142790 DBA Accession No.: 93-00842
Gene transfer - gene transmission by retro virus vector, *yeast* *artificial* *chromosome*, mouse zygote homologous *recombination*, Cre-*recombinase* method and Flp system (conference abstract)
AUTHOR: Wagner E F
CORPORATE SOURCE: Research Institute of Molecular Pathology (IMP), Dr Bohr-Gasse 7, A-1030, Vienna, Austria.
JOURNAL: Science (258, Suppl., 31-32) 1992 CODEN: SCIEAS
LANGUAGE: English
ABSTRACT: Applications and limitations of gene transfer techniques were discussed. The high efficiency of retro virus infection allows the introduction of genes into cells, e.g. hematopoietic cells. These viral systems provide a method for the generation of animal models for human blood diseases and for possible gene therapy applications. However, the use of *yeast* *artificial* *chromosomes* introduced into cells via DNA-lipid micelles, or the generation of large *transgenes* through homologous *recombination* in mouse zygotes, provide a much superior gene transfer system to viral vector systems. Gene transfer techniques are also being used to inactivate a given gene locus by gene targeting. Two new loss-of-function approaches have recently been developed: (1) using the Cre- *recombinase*; and (2) using the Flp system. These 2 new methods may allow tissue-specific and developmentally regulated gene inactivation in *transgenic* mice as a function of the site-specific *recombinase* action. (7 ref)

4/7/14 (Item 2 from file: 357)
139839 DBA Accession No.: 92-12331 PATENT
Detection of somatic and germline alterations of the human APC gene - colorectum cancer APC gene mutation detection using DNA probe or antibody; transgenic animal and yeast artificial chromosome; cancer, etc. diagnosis and prognosis
PATENT ASSIGNEE: Johns-Hopkins-Univ.; ICI; Univ.Utah; Cancer-Inst.Japan 1992
PATENT NUMBER: WO 9213103 PATENT DATE: 920806 WPI ACCESSION NO.: 92-284685 (9234)
PRIORITY APPLIC. NO.: US 741940 APPLIC. DATE: 910808

NATIONAL APPLIC. NO.: WO 92US376 APPLIC. DATE: 920116
LANGUAGE: English
ABSTRACT: A new method for diagnosis or prognosis of tumors comprises detecting somatic alteration of wild-type (wt) APC genes or mRNA in a tumor tissue by hybridization with a DNA probe or by observing shifts in electrophoretic mobility of single-stranded DNA. The following are claimed: (1) a method for retoring wt APC activity to a mutated cell involving double *recombination* with an active gene or active gene fragment, or administration of a molecule which mimics the activity of wt APC protein; (2) a pair of ssDNA primers for determination of APC gene sequence by the polymerase chain reaction; (3) a DNA probe complementary to human wt APC gene nucleotides 822-930, 931-1309. 1406-1545 or 1956-2256 for detection of altered APC genes; (4) a method for detecting cancer in a human; (5) a method for detecting predisposition to cancer, including detecting familial adenomatous polyposis (FAP) and Gardner Syndrome (GS); (6) human APC preparation; (7) APC-specific antibody preparation; (8) a *transgenic* animal carrying the APC allele from another species, optionally with a mutation; (9) cDNA and DNA; and (10) *yeast* *artificial* *chromosome* 37HG4. (132pp)

4/7/15 (Item 3 from file: 357)
135901 DBA Accession No.: 92-08393
Targeted alterations in yeast artificial chromosomes for inter-species gene transfer - Saccharomyces cerevisiae artificial chromosome plasmid pLNA and plasmid pLUNA vector for potential gene targeting and transgenic animal construction using large DNA insert
AUTHOR: Davies N P; Rosewell I R; +Brueggemann M
CORPORATE SOURCE: AFRC, Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, CB2 4AT, UK.
JOURNAL: Nucleic Acids Res. (20, 11, 2693-98) 1992 CODEN: NARHAD
LANGUAGE: English
ABSTRACT: In order to facilitate alterations of large DNA molecules for their introduction into mammal cells, the mechanism of site-specific modification in *yeast* *artificial* *chromosomes* (*YACs*) was characterized. New yeast integration vectors (plasmid pLNA and plasmid pLUNA) with dominant selectable marker genes allowed targeted integration into left (centromeric) and right (non-centromeric) *YAC* arms, as well as alterations to the human-derived insert DNA. In transformation experiments, integration proceeded exclusively by homologous *recombination* , although Saccharomyces cerevisiae AB1380 preferred linear ends of homology for predefined insertions. Targeted regions could be rescued to facilitate the cloning of internal human sequences and the identification of 5' and 3' *YAC*/insert borders. Integration of a neomycin-resistance gene into various parts of the *YAC* allows the transfer and stable integration of large DNA molecules into a variety of mammalian cells, including embryonic stem cells. The vectors should be useful e.g. in gene targeting and *transgenic* animal construction using large DNA fragments. (39 ref)

4/7/16 (Item 4 from file: 357)
107734 DBA Accession No.: 90-10425
Transfer of a yeast artificial chromosome carrying human DNA from Saccharomyces cerevisiae into mammalian cells - yeast spheroplast fusion with mouse fibroblast L-cell for neomycin-resistance gene transmission
AUTHOR: Pachnis V; Pevny L; Rothstein R; Costantini F

CORPORATE SOURCE: Department of Genetics and Development, Columbia University, 701 West 168th Street, New York, NY 10032, USA.
JOURNAL: Proc.Natl.Acad.Sci.U.S.A. (87, 13, 5109-13) 1990 CODEN: PNASA6
LANGUAGE: English
ABSTRACT: To test the feasibility of transferring yeast artificial chromosomes (YACs) into mammalian cells, a YAC carrying a 450 kb segment of human DNA was modified by insertion of a neomycin-resistance gene. YAC-containing Saccharomyces cerevisiae AB1380-HY-19-neo was grown to stationary phase and treated with Zymolase. The resulting spheroplasts were pelleted, and exponentially-growing L-cells were centrifuged onto the spheroplast pallet. The 2 pellets were mixed in the presence of 50% PEG 1500, and fused cells were selected for neomycin-resistance. A high percentage of resistant clones contained virtually intact YAC sequences as revealed by Alu fingerprint analysis and restriction enzyme analysis using pulsed-field gel electrophoresis. The YAC sequences were stably integrated into the mouse chromosomes, as shown by in situ hybridization and by the stability of G418 resistance. The results establish that large segments of the mammalian genome, cloned in yeast, can be efficiently transferred into cultured mammalian cells. The YAC transfer system may be applicable to embryonic stem cells leading to the generation of transgenic animals. (22 ref)

4/7/17 (Item 5 from file: 357)
106722 DBA Accession No.: 90-09413 PATENT
Production of antibodies from transgenic animals - chimeric antibody, monoclonal antibody production from transgenic animal by microinjection of foreign immunoglobulin DNA into germline and harvesting from cell or body fluid
PATENT ASSIGNEE: Med.Res.Counc.; Agric.Food-Res.Counc. 1990
PATENT NUMBER: WO 9004036 PATENT DATE: 900419 WPI ACCESSION NO.: 90-147847 (9019)
PRIORITY APPLIC. NO.: GB 8823869 APPLIC. DATE: 881012
NATIONAL APPLIC. NO.: WO 89GB1207 APPLIC. DATE: 891012
LANGUAGE: English
ABSTRACT: Ig production involves obtaining Ig from cells or body fluid of a transgenic animal which has had genetic material encoding at least part of a foreign Ig, or genetic material that is capable of rearranging to encode a repertoire of Igs, inserted into its germline. Ig is produced in a body fluid or secretion of the animal or in vitro from cells obtained from the animal. The inserted genetic material encodes the entire species-specific regions of an Ig, and foreign Ig to a particular antigen is produced in response to subsequent introduction of the antigen into the animal. The inserted genetic material is of human origin, encodes an entire Ig, and comprises a plasmid or cosmid, multiple plasmids or cosmids, a yeast artificial chromosome or a mammalian chromosome fragment. The genetic material is inserted by injection into fertilized eggs or embryonic stem cells. A foreign Ig obtained from a transgenic animals, and a transgenic animal, particularly a non-human mammal, are also new. Polyclonal antisera may be obtained from the transgenic animal following immunization, or monoclonal antibodies may be produced from its spleen cells. (26pp)

4/7/18 (Item 1 from file: 434)
12419530 Genuine Article#: LK345 Number of References: 18
Title: MECHANISM OF CHROMOSOMAL INTEGRATION OF TRANSGENES IN MICROINJECTED MOUSE EGGS - SEQUENCE-ANALYSIS OF GENOME-TRANSGENE AND TRANSGENE-TRANSGENE JUNCTIONS AT 2 LOCI

Author(s): HAMADA T; SASAKI H; SEKI R; SAKAKI Y
Corporate Source: UNIV TOKYO,INST MED SCI,MOLEC MED LAB,4-6-1 SHIROGANEOAI,MINATO KU/TOKYO 108//JAPAN/; UNIV TOKYO,INST MED SCI,MOLEC MED LAB,4-6-1 SHIROGANEOAI,MINATO KU/TOKYO 108//JAPAN/; KYUSHU UNIV,GENET INFORMAT RES LAB/FUKUOKA 812//JAPAN/
Journal: GENE, 1993, V128, N2 (JUN 30), P197-202
ISSN: 0378-1119
Language: ENGLISH Document Type: ARTICLE
Abstract: Production of transgenic animals is a key technique in modern biology, but the process of chromosomal integration of transgenes in microinjected eggs is still not fully understood. To gain information on the mechanisms involved in this process, we have cloned two transgene loci and their corresponding pre-integration sites and compared the junction sequences with the parental nucleotide (nt) sequences. No extensive DNA rearrangements were detected at these loci: only simple deletions (caused by the integration of the transgene concatemers) were present in the host genome. Analysis of three transgene-transgene junctions within the concatemers showed that 'nibbling' of ends (up to 3 nt) had occurred at some ends prior to joining. At all four genome-transgene junctions, short homologies of 1 to 3 nt were found, and at least three of these junctions were associated with the consensus sequence for topoisomerase-I cleavage sites. Moreover, three of the four integration junctions occurred in the terminal regions of the injected sequence, at positions only a few nt away from the ends. These results suggest that linear, but not circular, concatemers were preferentially integrated at their ends utilizing short homologies to the host genome.

4/7/19 (Item 2 from file: 434)
12348494 Genuine Article#: LE155 Number of References: 338
Title: MOLECULES AND COGNITION - THE LATTERDAY LESSONS OF LEVELS, LANGUAGE, AND LAC - EVOLUTIONARY OVERVIEW OF BRAIN STRUCTURE AND FUNCTION IN SOME VERTEBRATES AND INVERTEBRATES
Author(s): MIKLOS GLG
Corporate Source: AUSTRALIAN NATL UNIV,RES SCH BIOL SCI,CTR MOLECSTRUCT & FUNCT/CANBERRA/ACT 2601/AUSTRALIA/
Journal: JOURNAL OF NEUROBIOLOGY, 1993, V24, N6 (JUN), P842-890
ISSN: 0022-3034
Language: ENGLISH Document Type: REVIEW
Abstract: The characteristics of the nervous systems of a number of organisms in different phyla are examined at the *recombinant* DNA, protein, neuroanatomic, neurophysiological, and cognitive levels. Among the invertebrates, special attention is paid to the advantages as well as the shortcomings of the fly Drosophila melanogaster, the worm Caenorhabditis elegans, the honey bee Apis mellifera, the sea hare Aplysia californica, the octopus Octopus vulgaris, and the squid Loligo pealei. Among vertebrates, the focus is on Homo sapiens, the mouse Mus musculus, the rat Rattus norvegicus, the cat Felis catus, the macaque monkey Macaca fascicularis, the barn owl Tyto alba, and the zebrafish Brachydanio rerio. Vertebrate nervous systems have also been compared in fossil vs. extant organisms. I conclude that complex nervous systems arose in the Early Cambrian via a big bang that was underpinned by a modular method of construction involving massive pleiotropy of gene circuits. This rapidity of construction had enormous implications for the degrees of freedom that were subsequently available to evolving nervous systems. I also conclude that at the level of neuronal populations and interactions of neuropiles there is no model system

between phyla except at the basic macromolecular level. Further, I argue that to achieve a significant understanding of the functions of extant nervous systems we need to concentrate on fewer organisms in greater depth and manipulate genomes via *transgenic* technologies to understand the behavioral outputs that are possible from an organism. Finally, I analyze the concepts of ''perceptual categorization'' and ''information processing'' and the difficulties involved in the extrapolation of computer analogies to sophisticated nervous systems.

4/7/20 (Item 3 from file: 434)
12329697 Genuine Article#: LC720 Number of References: 41
Title: ALTERATION OF CAENORHABDITIS-ELEGANS GENE-EXPRESSION BY TARGETED TRANSFORMATION
Author(s): BROVERMAN S; MACMORRIS M; BLUMENTHAL T
Corporate Source: INDIANA UNIV,DEPT BIOL/BLOOMINGTON//IN/47405; INDIANA UNIV,DEPT BIOL/BLOOMINGTON//IN/47405
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1993, V90, N10 (MAY 15), P4359-4363
ISSN: 0027-8424
Language: ENGLISH Document Type: ARTICLE
Abstract: We have produced strains carrying a synthetic fusion of parts of two vitellogenin genes, vit-2 and vit-6, integrated into the Caenorhabditis elegans genome. In most of the 63 transformant strains, the plasmid sequences are integrated at random locations in the genome. However, in two strains the *transgene* integrated by homologous *recombination* into the endogenous vit-2 gene. In both cases the reciprocal exchange between the chromosome and the injected circular plasmid containing a promoter deletion led to switching of the plasmid-borne promoter and the endogenous promoter, with a reduction in vit-2 expression. Thus in nematodes, transforming DNA can integrate by homologous *recombination* to result in partial inactivation of the chromosomal locus. The simplicity of the event and its reasonably high frequency suggest that gene targeting by homologous *recombination* should be considered as a method for directed inactivation of C. elegans genes.

4/7/21 (Item 4 from file: 434)
11961131 Genuine Article#: JZ634 Number of References: 21
Title: NESTED CHROMOSOMAL FRAGMENTATION IN YEAST USING THE MEGANUCLEASE I-SCE-I - A NEW METHOD FOR PHYSICAL MAPPING OF EUKARYOTIC GENOMES
Author(s): THIERRY A; DUJON B
Corporate Source: INST PASTEUR,DEPT BIOL MOLEC,CNRS,URA 1149,UNITE GENET MOLEC LEVURES,25 RUE DR ROUX/F-75724 PARIS 15//FRANCE/
Journal: NUCLEIC ACIDS RESEARCH, 1992, V20, N21 (NOV 11), P5625-5631
ISSN: 0305-1048
Language: ENGLISH Document Type: ARTICLE
Abstract: We have developed a new method for the physical mapping of genomes and the rapid sorting of genomic libraries which is based on chromosome fragmentation by the meganuclease I-Sce I, the first available member of a new class of endonucleases with very long recognition sequences. I-Sce I allows complete cleavage at a single artificially inserted site in an entire genome. Sites can be inserted by homologous *recombination* using specific cassettes containing selectable markers or, at random, using transposons. This method has been applied to the physical mapping of chromosome XI (620 kb) of Saccharomyces cerevisi and to the sorting of a cosmid library. Our strategy has potential applications to various genome mapping projects.

A set of *transgenic* yeast strains carrying the I-Sce I sites at various locations along a chromosome defines physical intervals against which new genes, DNA fragments or clones can be mapped directly by simple hybridizations.

4/7/22 (Item 5 from file: 434)
11803078 Genuine Article#: JM207 Number of References: 60
Title: THYMOCYTE ACTIVATION AND DEATH - A MECHANISM FOR MOLDING THE T-CELL REPERTOIRE
Author(s): ZACHARCHUK CM; MERCEP M; ASHWELL JD
Corporate Source: NCI,BIOL RESPONSE MODIFIERS PROGRAM,BLDG 10,ROOM 13N-268/BETHESDA//MD/20892
Journal: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, 1991, V636, DEC (DEC 30), P52-70
ISSN: 0077-8923
Language: ENGLISH Document Type: ARTICLE

4/7/23 (Item 6 from file: 434)
11516055 Genuine Article#: HN012 Number of References: 107
Title: CHROMOSOMAL TRANSLOCATIONS IN LYMPHOID MALIGNANCIES REVEAL NOVEL PROTOONCOGENES
Author(s): KORSMEYER SJ
Corporate Source: WASHINGTON UNIV,SCH MED,HOWARD HUGHES MED INST,DEPT MED MOLEC MICROBIOL/ST LOUIS//MO/63110
Journal: ANNUAL REVIEW OF IMMUNOLOGY, 1992, V10, P785-807
Language: ENGLISH Document Type: REVIEW
Abstract: Chromosomal translocation within B and T cell malignancies has proven a rich source for proto-oncogenes. The obligate DNA breaks within immunoglobulin (Ig) and T cell receptor (TCR) loci are frequently the sites of recurrent translocations. Burkitt's lymphoma established the paradigm by introducing the myc oncogene from chromosome segment 8q24 into the Ig heavy chain gene locus at 14q32. Molecular cloning of an aberrant Ig rearrangement in follicular lymphoma revealed Bcl-2. Bcl-2 constitutes the first member of a new category of oncogenes: regulators of programmed cell death. Bcl-2 blocks apoptosis and maintains long-term immune responsiveness including B-cell memory. The PRAD1 gene of parathyroid adenomas appears to be the elusive Bcl-1 gene of t(11;14)(q13;q32) bearing lymphomas. It proves to be a novel G1 cyclin. Acute lymphoblastic leukemias (ALL) pre-B phenotype produce a E2A/PBX fusion protein that possesses the leucine zipper of E2A with the homeodomain of PBX. Two molecular forms of the BCR/ABL fusion protein are produced by the Philadelphia chromosome. A deregulated p210 tyrosine kinase is found in chronic myelogenous leukemia, while a p190 form predominates in Ph+ALL. In contrast, T-cell ALLs introduce a potpourri of genes into their T cell receptor loci. However, a common theme is emerging. These oncogenes (Ttg1, Ttg2, SCL, Lyl1, H0X11) all belong to classic families of transcription factors, possessing LIM domains, helix-loop-helix motifs, or homeodomains. Provocatively, these transcription factors are normally intended for lineages other than T cells. These genes have widened the horizons of both oncogenesis and normal development.

4/7/24 (Item 7 from file: 434)
11498274 Genuine Article#: HM420 Number of References: 20
Title: EFFICIENT GENERATION OF FUNCTIONAL *TRANSGENES* BY HOMOLOGOUS *RECOMBINATION* IN MURINE ZYGOTES

Author(s): PIEPER FR; DEWIT ICM; PRONK ACJ; KOOIMAN PM; STRIJKER R; KRIMPENFORT PJA; NUYENS JH; DEBOER HA
Corporate Source: GENEPHARMING EUROPE BV,POB 9502/2300 RA LEIDEN//NETHERLANDS/; LEIDEN UNIV/2300 RA LEIDEN//NETHERLANDS/
Journal: NUCLEIC ACIDS RESEARCH, 1992, V20, N6 (MAR 25), P1259-1264
Language: ENGLISH Document Type: ARTICLE
Abstract: To assess the feasibility of generating functional *transgenes* directly via homologous *recombination* between microinjected DNA fragments, three overlapping genomic DNA fragments, together constituting the human serum albumin (hSA) gene, were coinjected into murine zygotes. The resulting *transgenic* mice were analyzed for structure and expression of the *transgene*. All *transgenic* mice carried *recombined* hSA DNA fragments and 74% contained a reconstituted hSA gene. HSA expression could be detected in liver and serum in most (72%) of these animals. Only correctly sized hSA transcripts were observed. *Transgenic* hSA could not be distinguished from the human serum-derived protein by radioimmunoassay or Western blotting. The high frequency and accuracy of homologous *recombination* in murine zygotes reported here allows the efficient generation of relatively large *transgenes*.

4/7/25 (Item 8 from file: 434)
11459063 Genuine Article#: HK310 Number of References: 23
Title: CONSTRUCTION OF YEAST ARTIFICIAL CHROMOSOMES CONTAINING BARLEY DNA AND THE IDENTIFICATION OF CLONES CARRYING COPIES OF THE REPEATED ELEMENT BIS-1
Author(s): DUNFORD R; ROGNER UC
Corporate Source: CARLSBERG LAB,DEPT PHYSIOL,GAMLE CARLSBERG VEJ 10/DK-2500 COPENHAGEN//DENMARK/; CARLSBERG LAB,DEPT PHYSIOL,GAMLE CARLSBERG VEJ 10/DK-2500 COPENHAGEN//DENMARK/; RHEIN WESTFAL TH AACHEN,INST BOT/W-5100 AACHEN//GERMANY/
Journal: HEREDITAS, 1991, V115, N2, P133-138
Language: ENGLISH Document Type: ARTICLE
Abstract: *Yeast* *artificial* *chromosome* (*YAC*) cloning vectors allow the isolation and analysis of very large segments of DNA. Barley DNA was cleaved with the rare-cutting restriction enzyme MluI and fractionated according to size on a linear sucrose gradient or by pulsed field gel electrophoresis. DNA fragments of approximately 50-250 kb were ligated with the *YAC* vector pYAC-RC and transformed into yeast spheroplasts. The presence of *recombinant* *YACs* with barley DNA inserts was established, and a number of clones containing copies of the repeated element BIS-1 were isolated. These results show that *YAC* cloning techniques can be successfully applied to the analysis of the barley genome.

4/7/26 (Item 9 from file: 434)
11199292 Genuine Article#: GP895 Number of References: 25
Title: RECOGNITION OF BETA-2-MICROGLOBULIN-NEGATIVE (BETA-2M-) T-CELL BLASTS BY NATURAL-KILLER-CELLS FROM NORMAL BUT NOT FROM BETA-2M- MICE - NONRESPONSIVENESS CONTROLLED BY BETA-2M- BONE-MARROW IN CHIMERIC MICE
Author(s): HOGLUND P; OHLEN C; CARBONE E; FRANKSSON L; LJUNGGREN HG; LATOUR A; KOLLER B; KARRE K
Corporate Source: KAROLINSKA INST,DEPT TUMOR BIOL,BOX 60400/S-10401 STOCKHOLM 60//SWEDEN/; UNIV N CAROLINA,SCH MED,DEPT PATHOL/CHAPEL HILL//NC/27599
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1991, V88, N22, P10332-10336

Language: ENGLISH Document Type: ARTICLE
Abstract: The role of major histocompatibility complex (MHC) class I expression in control of the sensitivity of normal cells to natural killer (NK) cells was studied by the use of mutant mice made deficient for expression of beta-2-microglobulin (beta-2m) through homologous *recombination* in embryonal stem cells. T-cell blasts from beta-2m-deficient (beta-2m -/-) mice were killed by NK cells from normal mice in vitro, while beta-2m +/-blasts were resistant. The beta-2m defect also affected the NK effector cell repertoire: NK cells from beta-2m -/- mice failed to kill beta-2m -/- blasts, while they retained the ability to kill the prototype NK cell target lymphoma *YAC*-1, although at reduced levels. The inability to recognize beta-2m -/- blasts could be transferred with beta-2m -/- bone marrow to irradiated beta-2m-expressing mice. In contrast, the development of CD8+ T cells (deficient in beta-2m -/- mice) was restored in such chimera. These results indicate that loss of MHC class I/beta-2m expression is sufficient to render normal cells sensitive to NK cells, and that the same defect in the hemopoietic system of a mouse renders its NK cells tolerant to beta-2m-deficient but otherwise normal cells. In the beta-2m -/- mice, NK cells may be selected or educated by other bone marrow cells to tolerate the MHC class I deficiency. Alternatively, the specificity may be controlled directly by the class I molecules on the NK cells themselves.

4/7/27 (Item 10 from file: 434)
10946558 Genuine Article#: FU901 Number of References: 24
Title: GENERATION OF A NESTED SERIES OF INTERSTITIAL DELETIONS IN YEAST ARTIFICIAL CHROMOSOMES CARRYING HUMAN DNA
Author(s): CAMPBELL C; GULATI R; NANDI AK; FLOY K; HIETER P; KUCHERLAPATI RS
Corporate Source: YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT MOLEC GENET,1300 MORRIS PK AVE/BRONX//NY/10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT MOLEC GENET,1300 MORRIS PK AVE/BRONX//NY/10461; JOHNS HOPKINS UNIV,SCH MED,DEPT MOLEC BIOL & GENET/BALTIMORE//MD/21205; YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT CELLBIOL/BRONX//NY/10461
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1991, V88, N13, P5744-5748
Language: ENGLISH Document Type: ARTICLE
Abstract: We have generated a nested series of interstitial deletions in a fragment of human X chromosome-derived DNA cloned into a *yeast* *artificial* *chromosome* (*YAC*) vector. A yeast strain carrying the *YAC* was transformed with a linear *recombination* substrate containing at one end a sequence that is uniquely represented on the *YAC* and at the other end a truncated long interspersed repetitive element (LINE 1, or L1). Homologous *recombination* between the *YAC* and the input DNA resulted in a nested series of interstitial deletions, the largest of which was 500 kilobases. In combination with terminal deletions that can be generated through homologous *recombination*, the interstitial deletions are useful for mapping and studying gene structure-function relationships.

4/7/28 (Item 11 from file: 434)
10716639 Genuine Article#: FB629 Number of References: 32
Title: THE HUMAN HPRT GENE ON A YEAST ARTIFICIAL CHROMOSOME IS FUNCTIONAL WHEN TRANSFERRED TO MOUSE CELLS BY CELL-FUSION
Author(s): HUXLEY C; HAGINO Y; SCHLESSINGER D; OLSON MV
Corporate Source: WASHINGTON UNIV,SCH MED,DEPT GENET/ST LOUIS//MO/63110;

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    WASHINGTON UNIV,SCH MED,HOWARD HUGHES MED INST/ST LOUIS//MO/63110;
    WASHINGTON UNIV,SCH MED,DEPT MOLEC MICROBIOL/STLOUIS//MO/63110
Journal: GENOMICS, 1991, V9, N4, P742-750
Language: ENGLISH   Document Type: ARTICLE
?s homologous(5a)recombin?
      S5        0  HOMOLOGOUS(5A)RECOMBIN?
?s homolog?(s)recombin?
?s  homolog?(5n)recombin?
Processed  10 of  12 files ...
Completed processing all files
          338368  HOMOLOG?
          406296  RECOMBIN?
      S6   16755  HOMOLOG?(5N)RECOMBIN?
?s s6(s)s1 not s3
           16755  S6
            8296  S1
             138  S6(S)S1
              39  S3
      S7     129  S6(S)S1 NOT S3
?s s6(5n)s1 not s3
           16755  S6
            8296  S1
             129  S6(5N)S1
              39  S3
      S8     126  S6(5N)S1 NOT S3
?rd
>>>Duplicate detection is not supported for File 60.
>>>Duplicate detection is not supported for File 351.

>>>Records from unsupported files will be retained in the RD set.
...examined 50 records  (50)
...examined 50 records  (100)
...completed examining records
      S9      61  RD (unique items)
?t s9/7/1-40;t s9/3/41-61

 9/7/1     (Item 1 from file: 5)
10439939     BIOSIS Number: 96039939
  AN INTEGRATED APPROACH FOR IDENTIFYING AND MAPPING HUMAN GENES
  DAS GUPTA R; MORROW B; MARONDEL I; PARIMOO S; GOEI V L; GRUEN J; WEISSMAN
S; SKOULTCHI A; KUCHERLAPATI R
  DEP. MOL. GENET., ALBERT EINSTEIN COLL. MED., BRONX, NY 10461, USA.
  PROC NATL ACAD SCI U S A 90 (10). 1993.  4364-4368.   CODEN: PNASA
  Full Journal Title: Proceedings of the National Academy of Sciences of
the United States of America
  Language: ENGLISH
  We have developed a method for generating expressed-sequence maps of
human chromosomes. The method involves several steps that begin with
libraries of highly representative short cDNAs prepared by using random
oligomers as primers. The cDNA inserts are amplified by PCR with flanking
vector primers. Chromosomal region-specific cDNA packets are prepared by
hybridization of the cDNA inserts to DNA derived from yeast artificial
chromosomes (YACs) assigned to defined regions of human chromosomes. The
cDNA packets are cloned into yeast chromosome fragmentation vectors and
used for transformation of yeast bearing the *YAC* used for affinity
purification. Sequences in the cDNAs undergo *homologous* *recombination*
with the corresponding exons in the genomic DNA yielding a set of truncated
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YACs. Each unique truncation specifies the location of an exon in the YAC. Since all of the truncation events end with the same vector sequence, it is possible to rescue and sequence these ends to generate expressed sequence tags. The method couples rapid purification of region-specific cDNAs with precise mapping of their genes on YACs. Appropriately truncated YACs also provide easy access to gene regulatory sequences. We describe the feasibility of individual steps of the method using the factor IX (F9) gene as a model system and we present the mapping of several expressed sequences corresponding to a 330-kb YAC containing DNA from human chromosome 6p21. In addition, we obtained the sequence, including an intron-exon junction, flanking a particular truncation event.

9/7/2 (Item 2 from file: 5)
10131129 BIOSIS Number: 95131129
INCORPORATION OF COPY-NUMBER CONTROL ELEMENTS INTO *YEAST* *ARTIFICIAL* *CHROMOSOMES* BY TARGETED *HOMOLOGOUS* *RECOMBINATION*
SMITH D R; SMYTH A P; STRAUSS W M; MOIR D T
COLLABORATIVE RESEARCH INC., 1365 MAIN ST., WALTHAM, MA 02154, USA.
MAMM GENOME 4 (3). 1993. 141-147. CODEN: MAMGE
Language: ENGLISH
We have developed a pair of vectors for exchanging *yeast* *artificial* *chromosome* (*YAC*) arms by targeted *homologous* *recombination*. These conversion vectors allow the introduction of copy-number control elements into *YACs* constructed with pYAC4 or related vectors. YACs modified in this way provide an enriched source of DNA for genetic or biochemical studies. A LYS2 gene on the conversion vector provides a genetic selection for the modified YACs after transformation with appropriately prepared vector. A background of Lys+ clones that do not contain modified YACs is also present. However, clones with converted YACs can be distinguished from this background by counter-screening for loss of the original pYAC4 TRP1 arm (Trp- phenotype). The elimination of yeast replication origins (ARS elements) from the conversion vectors increased the frequency of Lys+ Trp- clones, but resulted in weaker amplification. Several YACs have been converted with these vectors, and the fate of the transformed DNA and of the resident YAC DNA has been systematically investigated.

9/7/3 (Item 3 from file: 5)
10050033 BIOSIS Number: 95050033
CREATION OF A YEAST ARTIFICIAL CHROMOSOME FRAGMENTATION VECTOR BASED ON LYSINE-2
LEWIS B C; SHAH N P; BRAUN B S; DENNY C T
DEP. PEDIATRICS, A2-312 MDCC, UCLA MED. CENT., LOS ANGELES, CALIF. 90024.
GENET ANAL TECH APPL 9 (3). 1992. 86-90. CODEN: GATAE
Language: ENGLISH
Yeast artificial chromosome (YAC) fragmentation vectors have proven to be useful reagents for mapping and modifying *YAC* clones through *homologous* *recombination* . Such vectors can be employed to remove a noncontiguous sequence present in chimeric YAC clones. However, previous fragmentation vectors have been based on auxotrophies not present in the background of most recombinant YAC libraries, which therefore makes them difficult to use. We have constructed an acentric YAC deletion vector pBCL that is fully compatible with AB 1380, the yeast host used in the majority of human total genomic YAC libraries. Transformation of three unrelated YACs with linearized pBCL yielded derivative clones with the expected phenotype at rates of 27%-49%. Analysis of a random cohort of these clones by pulsed-field gel electrophoresis and Southern blotting revealed single deleted structures in 31 of 34 clones. Using this vector, a nested set of

deletion derivatives can efficiently and reliably be generated from human recombinant YAC clones.

9/7/4 (Item 4 from file: 5)
9779250 BIOSIS Number: 44029250
HOMOLOGOUS *RECOMBINATION* GENE AMPLIFICATION AND DNA RESCUE IN *YACS*
ALMASAN A; PAULSON T; BRODY L; CLARKIN K; WAHL G M
SALK INST., SAN DIEGO, CALIF.
THE AMERICAN SOCIETY OF HUMAN GENETICS 42ND ANNUAL MEETING, SAN FRANCISCO, CALIFORNIA, USA, NOVEMBER 9-13, 1992. AM J HUM GENET 51 (4 SUPPL.). 1992. A233. CODEN: AJHGA
Language: ENGLISH

9/7/5 (Item 5 from file: 5)
9603006 BIOSIS Number: 94108006
RECOMBINATIONAL REPAIR OF DIVERGED DNAS A STUDY OF HOMOEOLOGOUS CHROMOSOMES AND MAMMALIAN YACS IN YEAST
RESNICK M A; ZGAGA Z; HIETER P; WESTMORELAND J; FOGEL S; NILSSON-TILLGREN T
LAB. MOLECULAR GENETICS, NATL. INST. ENVIRON. HEALTH SCI., RESEARCH TRIANGLE PARK, NC 27709.
MOL GEN GENET 234 (1). 1992. 65-73. CODEN: MGGEA
Full Journal Title: Molecular & General Genetics
Language: ENGLISH
Recombinational repair is the means by which DNA double-strand breaks (DSBs) are repaired in yeast. DNA divergence between chromosomes was shown previously to inhibit repair in diploid G1 cells, resulting in chromosome loss at low nonlethal doses of ionizing radiation. Furthermore, 15-20% divergence prevents meiotic recombination between individual pairs of Saccharomyces cerevisiae and S. carlsbergensis chromosomes in an otherwise S. cerevisiae background. Based on analysis of the efficiency of DSB-induced chromosome loss and direct genetic detection of intragenic recombination, we conclude that limited DSB recombinational repair can occur between homologous chromosomes. There is no difference in loss between a repair-proficient Pms+ strain and a mismatch repair mutant, pms1. Since DSB recombinational repair is tolerant of diverged DNAs, this type of repair could lead to novel genes and altered chromosomes. The sensitivity to DSB-induced loss of 11 individual yeast artificial chromosomes (YACs) containing mouse or human (chromosome 21 or HeLa) DNA was determined. *Recombinational* repair between a pair of *homologous* HeLa *YACs* appears as efficient as that between homologous yeast chromosomes in that there is no loss at low radiation doses. Single YACs exhibited considerable variation in response although the response for individual YACs was highly reproducible. Based on the results with the yeast homologous chromosomes, we propose that the potential exists for intra-YAC recombinational repair between diverged repeat DNA and that the extent of repair is dependent upon the amount of repeat DNA and the degree of divergence. The sensitivity of YACs containing mammalian DNA to ionizing radiation-induced loss may thus be an indicator of the extent of repeat DNA.

9/7/6 (Item 6 from file: 5)
9579814 BIOSIS Number: 94084814
TARGETED INTEGRATION OF NEOMYCIN INTO YEAST ARTIFICIAL CHROMOSOMES YACS FOR TRANSFECTION INTO MAMMALIAN CELLS
RILEY J H; MORTEN J E N; ANAND R
ICI PHARMACEUTICALS, BIOTECHNOL. DEP., ALDERLEY PARK, MACCLESFIELD, CHESHIRE SK10 4TG.

NUCLEIC ACIDS RES 20 (12). 1992. 2971-2976. CODEN: NARHA
Full Journal Title: Nucleic Acids Research
Language: ENGLISH

Vectors have been constructed for the introduction of the neomycin resistance gene (neo) into the left arm, right arm or human insert DNA of *yeast* *artificial* *chromosomes* (*YACs*) by *homologous* *recombination* . These vectors contain a yeast selectable marker Lys-2, i.e. the .alpha.-aminoadipidate reductase gene, and a mammalian selection marker, neo, which confers G418 resistance. The vectors can be used to modify YAC library construction, AB1380. Specific targeting can be carried out by transfection of restriction endonuclease treated linear plasmids, with highly specific recombinogenic ends, into the YAC containing yeast cells. Analysis of targeted YACs confirmed that all three vectors can target correctly in yeast. Introduction of one of the targeted YACs into V79 (Chinese hamster fibroblast) cells showed complete and intact transfer of the YAC.

9/7/7 (Item 7 from file: 5)
9545726 BIOSIS Number: 94050726

A YEAST ARTIFICIAL CHROMOSOME CONTIG ENCOMPASSING THE TYPE 1 NEUROFIBROMATOSIS GENE

MARCHUK D A; TAVAKKOL R; WALLACE M R; BROWNSTEIN B H; TAILLON-MILLER P; FONG C-T; LEGIUS E; ANDERSEN L B; GLOVER T W; COLLINS F S

DEP. RADIATION ONCOLOGY, DIVISION CANCER BIOLOGY, UNIVERSITY MICHIGAN MEDICAL SCHOOL, ANN ARBOR, MICH. 48109.

GENOMICS 13 (3). 1992. 672-680. CODEN: GNMCE
Full Journal Title: Genomics
Language: ENGLISH

The yeast artificial chromosome (YAC) system (Burke et al., 1987, Science 236: 806-812) allows the direct cloning of large regions of the genome. A YAC contig map of approximately 700 kb encompassing the region surrounding the type 1 neurofibromatosis (NF1) locus on 17q11.2 has been constructed. A single *YAC* containing the entire NF1 locus has been constructed by *homologous* *recombination* in yeast. In the process of contig construction a novel method of *YAC* end rescue has been developed by YAC circularization in yeast and plasmid rescue in bacteria. YACs containing homology to the NF1 region but mapping to another chromosome have also been discovered. Sequences of portions of the homologous locus indicate that this other locus is a nonprocessed pseudogene.

9/7/8 (Item 8 from file: 5)
9055548 BIOSIS Number: 93040548

HIGH-EFFICIENCY YEAST ARTIFICIAL CHROMOSOME FRAGMENTATION VECTORS

PAVAN W J; HIETER P; SEARS D; BURKHOFF A; REEVES R H

PHYSIOL. 202, JOHNS HOPKINS UNIV. SCH. MED., BALTIMORE, MD. 21205.

GENE (AMST) 106 (1). 1991. 125-128. CODEN: GENED
Full Journal Title: GENE (Amsterdam)
Language: ENGLISH

Chromosome fragmentation vectors (CFVs) are used to create deletion derivatives of large fragments of human DNA cloned as yeast artificial chromosomes (*YACs*). CFVs target insertion of a telomere sequence into the *YAC* via *homologous* *recombination* with Alu repetitive elements. This event results in the loss of all *YAC* sequences distal to the site of integration. A new series of CFVs has been developed. These vectors target fragmentation to both Alu and LINE human repetitive DNA elements. Recovery of deletion derivatives is ten- to 20-fold more efficient with the new vectors than with those described previously.

9/7/9 (Item 9 from file: 5)
8827049 BIOSIS Number: 42052049
RECONSTRUCTION AND FURTHER STUDY OF THE 2.4 MB DMD GENE BY *HOMOLOGOUS* *YAC* *RECOMBINANT*
VAN OMMEN G J B; DEN DUNNEN J T; GROOTSCHOLTEN P M; MONACO A P; ANAND R; BENTLEY D; STEENSMA Y
DEP. HUMAN GENETICS, LEIDEN UNIV., NETH.
PROCEEDINGS OF THE 8TH INTERNATIONAL CONGRESS OF HUMAN GENETICS, WASHINGTON, D.C., USA, OCTOBER 6-11, 1991. AM J HUM GENET 49 (4 SUPPL.). 1991. 4. CODEN: AJHGA
Language: ENGLISH

9/7/10 (Item 10 from file: 5)
8646850 BIOSIS Number: 92111850
VECTORS FOR INSERTING SELECTABLE MARKERS IN VECTOR ARMS AND HUMAN DNA INSERTS OF YEAST ARTIFICIAL CHROMOSOMES YACS
SRIVASTAVA A K; SCHLESSINGER D
DEP. MOLECULAR MICROBIOL., WASH. UNIV. SCH. MED., ST. LOUIS, MO. 63110, USA.
GENE (AMST) 103 (1). 1991. 53-60. CODEN: GENED
Full Journal Title: GENE (Amsterdam)
Language: ENGLISH
To facilitate studies of gene expression and homologous recombination, plasmids have been developed which permit the insertion of neomycin resistance-encoding gene (NmR) into either the human DNA insert or the vector arm of a yeast artificial chromosome (YAC). To integrate into the YAC arm, the plasmid pRV1 contains a LYS2 (encoding .alpha.-aminoadipate reductase) gene for selection in the yeast host, and a NmR gene for subsequent selection after transfection of mammalian cells. These two sequences are bracketed by fragments of the URA3 gene (encoding orotidine-5'-phosphate decarboxylase) that can disrupt the URA3 gene in the *YAC* arm by *homologous* *recombination* yeast. To integrate a selectable marker into the insert, the plasmid pRV2 contains a NmR gene and an intact copy of the URA3 gene, bracketed by segments of an L1 (LINEs) repetitive element. In this case, the vector has been designed for use with YACs that have already been fitted in the vector arm with a different marker (ie., TK) that has disrupted the URA3 gene in the vector arm. Selection is for the restoration of URA3 gene activity attendant on recombination into an L1 element in the YAC insert. Use of the vectors is illustrated with a YAC clone containing ribosomal DNA.

9/7/11 (Item 11 from file: 5)
8646837 BIOSIS Number: 92111837
INTEGRATIVE SELECTION OF HUMAN CHROMOSOME-SPECIFIC YEAST ARTIFICIAL CHROMOSOMES
PAVAN W J; REEVES R H
DEP. PHYSIOL., P202, JOHNS HOPKINS UNIVERSITY SCH. MED., 725 NORTH WOLFE STREET, BALTIMORE, MD. 21205.
PROC NATL ACAD SCI U S A 88 (17). 1991. 7788-7791. CODEN: PNASA
Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America
Language: ENGLISH
Human specific "integrative selection vectors" (ISVs) were designed to optimize integration of a yeast-selectable marker specifically into yeast artificial chromosomes (YACs) derived from human but not mouse DNA. ISVs were transformed into a YAC genomic library constructed from DNA of a

human-mouse somatic cell hybrid containing chromosome 21 (HSA21) as the only human chromosome. One percent of the yeast in the original library contained HSA21-derived YACs; between 45% and 54% of the yeast recovered after transformation with ISV vectors contained human YACs. Integrative selection provides a rapid means of obtaining a highly enriched population of human chromosome-specific YACs by eliminating the labor-intensive steps of isolating and screening primary transformants. The procedure is biased towards the selection of *YACs* that contain a large number of targets for *homologous* *recombination*; thus, libraries constructed by this procedure will be composed primarily of the largest YACs in the population.

9/7/12 (Item 12 from file: 5)
8599445 BIOSIS Number: 92064445
GENERATION OF A NESTED SERIES OF INTERSTITIAL DELETIONS IN YEAST ARTIFICIAL CHROMOSOMES CARRYING HUMAN DNA
CAMPBELL C; GULATI R; NANDI A K; FLOY K; HIETER P; KUCHERLAPATI R S
DEP. MOL. GENETICS, ALBERT EINSTEIN COLLEGE MED., 1300 MORRIS PARK AVE., BRONX, N.Y. 10461.
PROC NATL ACAD SCI U S A 88 (13). 1991. 5744-5748. CODEN: PNASA
Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America
Language: ENGLISH
We have generated a nested series of interstitial deletions in a fragment of human X chromosome-derived DNA cloned into a yeast artificial chromosome (YAC) vector. A yeast strain carrying the YAC was transformed with a linear recombination substrate containing at one end a sequence that is uniquely represented on the YAC and at the other end a truncated long interspersed repetitive element (LINE 1, or L1). *Homologous* *recombination* between the *YAC* and the input DNA resulted in a nested series of interstital deletions, the largest of which was 500 kilobases. In combination with terminal deletions that can be generated through homologous recombination, the interstitial deletions are useful for mapping and studying gene structure-function relationship.

9/7/13 (Item 13 from file: 5)
8197807 BIOSIS Number: 91118807
DETECTION OF *HOMOLOGOUS* *RECOMBINATION* BETWEEN *YEAST* *ARTIFICIAL* *CHROMOSOMES* WITH OVERLAPPING INSERTS
CELLINI A; LACATENA R M; TOCCHINI-VALENTINI G P
ISTITUTO DI BIOL. CELLULARE, CNR, 00137 ROME, ITALY.
NUCLEIC ACIDS RES 19 (5). 1991. 997-1000. CODEN: NARHA
Full Journal Title: Nucleic Acids Research
Language: ENGLISH
We have developed a system which facilitates the detection of recombination between Yeast Artificial Chromosomes (YAC's) carrying homologous inserts. The system consists of a classical YAC vector, a new YAC vector and two appropriately labelled yeast strains of opposite mating type. The new *YAC* vector differs in markers from the canonical *YAC* vector. To test whether *homologous* *recombination* takes place, phage lambda DNA was cloned in the two vectors to provide a region of homology. The two constructs were then introduced into yeast strains of opposite mating type in which the endogenous genes for the selective markers present in the vectors are not expressed. Artificial chromosomes obtained by meiotic recombination are detected in the spores resulting from the mating.

9/7/14 (Item 14 from file: 5)
8197572 BIOSIS Number: 91118572

STABLE INTEGRATION AND EXPRESSION IN MOUSE CELLS OF YEAST ARTIFICIAL CHROMOSOMES HARBORING HUMAN GENES

ELICEIRI B; LABELLA T; HAGINO Y; SRIVASTAVA A; SCHLESSINGER D; PILIA G; PALMIERI G; D'URSO M

DEP. MOLCULAR MICROBIOL. CENT. GENETICS MED., WASHINGTON UNIV. SCH. MED., ST. LOUIS, MO. 63110.

PROC NATL ACAD SCI U S A 88 (6). 1991. 2179-2183. CODEN: PNASA

Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America

Language: ENGLISH

We have developed a way to fit yeast artificial chromosomes (YACs) with markers that permit the selection of stably transformed mammalian cells, and have determined the fate and expression of such YACs containing the genes for human ribosomal RNA (rDNA) or glucose-6-phosphate dehydrogenase (G6PD). The *YACs* in the yeast cell are "retrofitted" with selectable markers by *homologous* *recombination* with the URA3 gene of one vector arm. The DNA fragment introduced contains a LYS2 marker selective in yeast and a thymidine kinase (TK) marker selective in TK-deficient cells, bracketed by portions of the URA3 sequence that disrupt the endogenous gene during the recombination event. Analyses of transformed L-M TK- mouse cells showed that YACs containing rDNA or G6PD were incorporated in essentially intact form into the mammalian cell DNA. for G6PD, a single copy of the transfected YAC was found in each of two transformants analyzed and was fully expressed, producing the expected human isozyme as well as the heterodimer composed of the human gene product and the endogenous mouse gene product.

9/7/15 (Item 15 from file: 5)
7718696 BIOSIS Number: 90086696

MODIFICATION AND TRANSFER INTO AN EMBRYONAL CARCINOMA CELL LINE OF A 360-KILOBASE HUMAN-DERIVED YEAST ARTIFICIAL CHROMOSOME

PAVAN W J; HIETER P; REEVES R H

DEV. GENET. LAB., DEP. PHYSIOL., JOHNS HOPKINS UNIV. SCH. MED., BALTIMORE, MD. 21205-2196.

MOL CELL BIOL 10 (8). 1990. 4163-4169. CODEN: MCEBD

Full Journal Title: Molecular and Cellular Biology

Language: ENGLISH

A neomycin resistance cassette was integrated into the human-derived insert of a 360-kilobase *yeast* *artificial* *chromosome* (*YAC*) by targeting *homologous* *recombination* to Alu repeat sequences. The modified *YAC* was transferred into an embryonal carcinoma cell line by using polyethylene glycol-mediated spheroplast fusion. A single copy of the human sequence was introduced intact and stably maintained in the absence of selection for over 40 generations. A substantial portion of the yeast genome was retained in hybrids in addition to the YAC. Hybrid cells containing the YAC retained the ability to differentiate when treated with retinoic acid. This approach provides a powerful tool for in vitro analysis because it can be used to modify any human DNA cloned as a YAC and to transfer large fragments of DNA intact into cultured mammalian cells, thereby facilitating functional studies of genes in the context of extensive flanking DNA sequences.

9/7/16 (Item 16 from file: 5)
7442114 BIOSIS Number: 89093133

GENERATION OF DELETION BY TARGETED TRANSFORMATION OF HUMAN-DERIVED YEAST ARTIFICIAL CHROMOSOMES

PAVAN W J; HIETER P; REEVES R H

DEP. PHYSIOL., JOHNS HOPKINS UNIV. SCH. MED., BALTIMORE, MD. 21205.
PROC NATL ACAD SCI U S A 87 (4). 1990. 1300-1304. CODEN: PNASA
Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America
Language: ENGLISH
Mammalian DNA segments cloned as yeast artificial chromosomes (YACs) can be manipulated by DNA-mediated transformation when placed in an appropriate yeast genetic background. A "fragmenting vector" has been developed that can introduce a yeast telomere and selectable marker into human-derived *YACs* at specific sites by means of *homologous* *recombination*, deleting all sequences distal to the recombination site. A powderful application of the method uses a human Alu family repeat sequence to target recombination to multiple independent sites on a human-derived YAC. Sets of deletion derivatives generated by this procedure greatly facilitate restriction mapping of large genomic segments. Targeting recombination with single copy sequences, such as cDNAs, will have many additional applications. This approach establishes a paradigm for manipulation and characterization of mammalian DNA segments cloned a YACs.

9/7/17 (Item 1 from file: 6)
1614935 NTIS Accession Number: DE92009806/XAB
New method employing homologous recombination and YAC rescue to expedite gap filling long range mapping. Progress report
Salk Inst. for Biological Studies, La Jolla, CA.
Corp. Source Codes: 088326000; 5643850
Sponsor: Department of Energy, Washington, DC.
Report No.: DOE/ER/61144-T2
1991 7p
Languages: English
Journal Announcement: GRAI9218; ERA9236
Sponsored by Department of Energy, Washington, DC.
NTIS Prices: PC A02/MF A01
Country of Publication: United States
Contract No.: FG03-91ER61144
We have embarked on three areas of research relevant to the telomere rescue strategy mediated by homologous recombination described in this proposal. First, we have constructed the telomere rescue vector. Second, we have carried out tests in yeast and mammalian cells to ascertain whether the various crucial components function. Finally, we have begun to develop the molecular reagents required to target the telomeric regions of chromosome 16. The specific progress in each area is described briefly below.

9/7/18 (Item 1 from file: 73)
8904310 EMBASE No: 93208099
Measurement of *recombinant* frequencies between two *homologous* DNA segments embedded in a *YAC* vector
Yasui H.; Kurosawa Y.
Inst. Comprehensive Medical Science, Fujita Health University, Toyoake 740-11 Japan
GENE (Netherlands) , 1993, 129/1 (135-139) CODEN: GENED ISSN: 0378-1119 ADONIS ORDER NUMBER: 0378111993003248
LANGUAGES: English SUMMARY LANGUAGES: English
We measured the frequencies of recombination in a yeast host between two homologous segments of DNA that had been inserted with the same polarity in a yeast artificial chromosome (YAC) vector. Three kinds of YAC clones were constructed in which the gene encoding neomycin(Nm) resistance was

sandwiched between two homologous segments of DNA, such as the IS3 elements of Escherichia coli or human Alu sequences. Frequencies of homologous recombination in yeast were measured in terms of loss of resistance to Nm. In the case of IS3 fragments, homologous recombination between them did occur at a relatively high frequency (5 x 10-4). In contrast, recombination between two Alu sequences did not occur at a detectable level during a 30-day incubation. Thus, the frequency was less than 10-5. These results indicate that the Alu sequences do not sufficiently promote the frequency of recombination between two homologous fragments in yeast as to induce rearrangements of DNA in a substantial fraction of YAC clones in libraries.

9/7/19 (Item 2 from file: 73)
8397026 EMBASE No: 92072917
Yeast artificial chromosomes (YACs) and the analysis of complex genomes
Anand R.
Biotechnology Department, ICI Pharmaceuticals, Alderley Park, Macclesfield SK10 4TG United Kingdom
TRENDS BIOTECHNOL. (United Kingdom) , 1992, 10/1-2 (35-40) CODEN: TRBID ISSN: 0167-9430 ADONIS ORDER NUMBER: 0167943092000112
LANGUAGES: English SUMMARY LANGUAGES: English
The development of yeast artificial chromosome (YAC) cloning vectors capable of carrying several hundred kilobase-pairs of DNA insert has greatly facilitated the study of complex genomes, and the cloning of large genes as single fragments. In addition, the ability to manipulate *YAC* sequences by *homologous* *recombination* makes this system extremely useful for the generation of disease models.

9/7/20 (Item 3 from file: 73)
8255336 EMBASE No: 91286468
Rescue of end fragments of *yeast* *artificial* *chromosomes* by *homologous* *recombination* in yeast
Hermanson G.G.; Hoekstra M.F.; McElligott D.L.; Evans G.A.
Molecular Genetics Laboratory, The Salk Institute, PO Box 85800, San Diego, CA 92138 USA
NUCLEIC ACIDS RES. (United Kingdom) , 1991, 19/18 (4943-4948) CODEN: NARHA ISSN: 0305-1048
LANGUAGES: English
Yeast artificial chromosomes (YACs) provide a powerful tool for the isolation and mapping of large regions of mammalian chromosomes. We developed a rapid and efficient method for the isolation of DNA fragments representing the extreme ends of YAC clones by the insertion of a rescue plasmid into the *YAC* vector by *homologous* *recombination*. Two rescue vectors were constructed containing a yeast LYS2 selectable gene, a bacterial origin of replication, an antibiotic resistance gene, a polylinker containing multiple restriction sites, and a fragment homologous to one arm of the pYAC4 vector. The 'end-cloning' procedure involves transformation of the rescue vector into yeast cells carrying a YAC clone, followed by preparation of yeast DNA and transformation into bacterial cells. The resulting plasmids carry end-specific DNA fragments up to 20kb in length, which are suitable for use as hybridization probes, as templates for direct DNA sequencing, and as probes for mapping by fluorescence in situ hybridization. These vectors are suitable for the rescue of end-clones from any YAC constructed using a pYAC-derived vector. We demonstrate the utility of these plasmids by rescuing YAC-end fragments from a human YAC library.

9/7/21 (Item 4 from file: 73)

7649961 EMBASE No: 90080639
Generation of deletion derivatives by targeted transformation of human-derived yeast artificial chromosomes
Pavan W.J.; Hieter P.; Reeves R.H.
Department of Physiology, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205 USA
PROC. NATL ACAD. SCI. U. S. A. (USA) , 1990, 87/4 (1300-1304) CODEN: PNASA ISSN: 0027-8424
LANGUAGES: English
Mammalian DNA segments cloned as yeast artificial chromosomes (YACs) can be manipulated by DNA-mediated transformation when placed in an appropriate yeast genetic background. A 'fragmenting vector' has been developed that can introduce a yeast telomere and selectable marker into human-derived *YACs* at specific sites by means of *homologous* *recombination*, deleting all sequences distal to the recombination site. A powerful application of the method uses a human Alu family repeat sequence to target recombination to multiple independent sites on a human-derived YAC. Sets of deletion derivatives generated by this procedure greatly facilitate restriction mapping of large genomic segments. Targeting recombination with single copy sequences, such as cDNAs, will have many additional applications. This approach establishes a paradigm for manipulation and characterization of mammalian DNA segments cloned as YACs.

9/7/22 (Item 1 from file: 76)
1712085 82002737965
Reconstruction of the 2.4 Mb human DMD-gene by homologous YAC recombination.
Dunnen, J.T.D.; Grootscholten, P.M.; Dauwerse, J.G.; Walker, A.P.; Monaco, A.P.; Butler, R.; Anand, R.; Coffey, A.J.; Van Ommen, G.J.B.; et al.
Dep. Hum. Genet., Leiden Univ., Wassenaarseweg 72, 2333 AL Leiden, Netherlands
HUM. MOL. GENET.; 1(1), pp. 19-28 1992
Language: English Summary Language: English
Document Type: Journal article-original research
Subfile: 07 Genetics Abstracts; 12 Human Genome Abstracts
The human dystrophin gene, mutations of which cause Duchenne and Becker muscular dystrophy, measures 2.4 Mb. This size seriously limits its cloning as a single DNA fragment and subsequent in-vitro expression studies. We have used stepwise in-vivo recombination between overlapping yeast artificial chromosomes (YACs) to reconstruct the dystrophin gene. The recombinant YACs are mitotically stable upon propagation in haploid yeast cells. In contrast, specific combinations of YACs display a remarkable mitotic and meiotic instability in diploid cells. Non-disjunction is rare for overlapping YACs, but increases upon sporulation of diploid cells containing non-overlapping molecules.

9/7/23 (Item 1 from file: 144)
10260864 PASCAL No.: 92-0466776
Reconstruction of the 2.4 Mb human DMD-gene by *homologous* *YAC* *recombination*
DEN DUNNEN J T; GROOTSCHOLTEN P M; DAUWERSE J G; WALKER A P; MONACO A P; BUTLER R; ANAND R; COFFEY A J; BENTLEY D R; STEENSMA H Y; VAN OMMEN G J B
Leiden univ., dep. human gen., 2333 AL Leiden, Netherlands
Journal: Human molecular genetics, 1992, 1 (1) 19-28
Availability: INIST-22540; 354000028895830040
No. of Refs.: 52 ref.

Document Type: P (Serial) ; A (Analytic)
Country of Publication: United Kingdom
Language: English

9/7/24 (Item 2 from file: 144)
10197548 PASCAL No.: 92-0403342
Targeted integration of neomycin into yeast artificial chronmosomes (YACs) for transfection into mammalian cells
RILEY J H; MORTEN J E N; ANAND R
Biotechnology dep., ICI pharmacueticals, Macclesfield Cheshire SK10 4TG, United Kingdom
Journal: Nucleic acids research, 1992, 20 (12) 2971-2976
ISSN: 0305-1048 CODEN: NARHAD Availability: INIST-15615; 354000028865480070
No. of Refs.: 19 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: United Kingdom
Language: English
Vectors have been constructed for the introduction of the neomycin resistance gene (neo) into the left arm, right arm or human insert DNA of *yeast* *artificial* *chromosomes* (*YACs*) by *homologous* *recombination* . These vectors contain a yeast selectable marker Lys-2, i.e. the alpha -aminoadipidate reductase gene, and a mammalian selection marker, neo, which confers G418 resistance. The vectors can be used to modify YACs in the most commonly used yeast strain for YAC library construction, AB1380. Specific targeting can be carried out by transfection of restriction endonuclease treated linear plasmids, with highly specific recombinogenic ends, into the YAC containing yeast cells

9/7/25 (Item 1 from file: 265)
0138799 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 5R21HG00463-02 AGENCY CODE: CRISP
Genetic manipulation of yeast artificial chromosomes
PRINCIPAL INVESTIGATOR: ELLEDGE, STEPHEN J
ADDRESS: BAYLOR COLLEGE OF MEDICINE ONE BAYLOR PLAZA HOUSTON, TX 77030
PERFORMING ORG.: BAYLOR COLLEGE OF MEDICINE, HOUSTON, TEXAS
SPONSORING ORG.: NATIONAL CENTER FOR HUMAN GENOME RESEARCH
FY : 93 FUNDS: $137,280 TYPE OF AWARD: Noncompeting Continuation (Type 5)
SUMMARY: The ability to isolate and maintain large fragments of DNA as artificial chromosomes in yeast (YACs) has ushered in a new era in the analysis of large genomes. Advances in this technology will be critical to the accomplishment of the goals put forth for the Human Genome Initiative. Improvements in the areas of generation and analysis of the libraries of human DNA will greatly facilitate progress towards meeting the goal of a contiguous ordered array of clones representing the entire human genome, the starting point for sequencing the genome. This proposal takes advantage of several useful features of yeast biology to advance these goals, in particular the extremely efficient pathway for homologous recombination. We propose to develop a set of YAC vectors that will allow several different types of genetic manipulation of YACs including chromosome walking using a genetic strategy. Walking will be accomplished by mating of a given YAC clone in yeast to a library of YACs in yeast made with suitable genetically marked vectors. Colonies containing pairs of YACs will be tested for the ability to undergo homologous recombination, allowing determination of those YACs containing overlapping stretches of genomic DNA. A novel in vivo method for the directed cloning of particular defined segments of genomic

DNA will be explored. This strategy called "DNA capture" utilizes the ability of yeast to gap repair DNA between two distantly located but genetically linked probes using total uncut genomic DNA as its repair template. The probes, attached to YAC vectors, are introduced into the yeast cells along with uncut chromosomal DNA via transformation, followed by selection for stable inheritance of the YAC markers. This strategy will also be used to explore the non-enzymatic production of genomic YAC libraries by using repetitive DNA sequences instead of unique sequences as probes for the DNA capture. A strategy for the genetic identification of YACs homologous to unique non-repetitive probes win be developed. This method will utilize homologous recombination enhanced by induced double-stranded breaks in DNA to activate expression of a defective gene on one arm of a YAC clone. A variation of this technique, " cDNA capture", can also be employed to identify all cDNAs in a library that are homologous to a particular YAC, a need that will arise later in the development of the Human Genome Initiative. The use of cre-lox-mediated site-specific recombination in yeast will be investigated for two purposes. First, efficient removal of CENs and selectable markers from YACs will be explored to facilitate the genetic selections for homologous recombination designed earlier in this proposal. Ibis will be entail developing a negative selection against lox sites. Secondly, circularization of YACs via site-specific recombination will be examined as a tool to alter the topological state of YACs to facilitate purification of YAC DNA away from yeast chromosomal DNA. Circularization of YACs will also be used to generate molecules that can successfully replicate in E. coli, a second method facilitating the isolation of YAC DNA.

9/7/26 (Item 2 from file: 265)
0134387 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 5R01HG00380-03 AGENCY CODE: CRISP
Human genome dissection by homologous recombination
PRINCIPAL INVESTIGATOR: KUCHERLAPATI, RAJU S
ADDRESS: ALBERT EINSTEIN COLLEGE OF MED 1300 MORRIS PARK AVENUE BRONX, NY 10461
PERFORMING ORG.: YESHIVA UNIVERSITY, NEW YORK, NEW YORK
SPONSORING ORG.: NATIONAL CENTER FOR HUMAN GENOME RESEARCH
FY : 93 FUNDS: $333,873 TYPE OF AWARD: Noncompeting Continuation (Type 5)
SUMMARY: DESCRIPTION: (Adapted from Investigator's Abstract) The goal of this proposal is to utilize homologous recombination strategies to generate defined interstitial and terminal deletions of individual human chromosomes. A nested series of interstitial and terminal deletions would aid in generating deletions in the human X-chromosome and using them to map several markers on this chromosome. Interstitial deletions will be generated first in YACs containing human DNA. A recombination vector containing a fragment of human clotting factor IX (F9) at one end and a human repetitive element (LINE 1) at the other with selectable markers in between (HIS3 for yeast and neo for mammalian cells) will be introduced into yeast containing a 650 kb YAC which has the complete F9 gene. Homologous recombination (HR) between the vector and its target sequences in the YAC would result in deletions extending from the F9 to each of several L1 sequences located on the chromosome. Similar experiments will be conducted in rodent-human somatic cell hybrids containing an intact human X-chromosome or part of it translocated onto the tip of a rodent chromosome. HR events will be isolated by selection for G418-resistant colonies followed by screening by the polymerase chain reaction (PCR). Terminal deletions in the human X-chromosome will be generated by targeting

the L1 repeat elements with a vector containing a dominant selectable marker and human telomeric sequences. Homologous recombination of the introduced DNA with each of its homologous sequences on the X-chromosome will yield a nested series of terminal deletions. The deletions that are generated will be characterized at the molecular as well as cytological level. An ordered set of deletions of increasing size will be used to map markers on the X-chromosome. The map that is generated will be compared to existing maps. If successful, this strategy could be employed to generate similar deletions in any human chromosome.

9/7/27 (Item 3 from file: 265)
0107653 DIALOG FILE NO. 265/266 FEDERAL RESEARCH IN PROGRESS
IDENTIFYING NO.: 3P01HG00202-03S2 0003 AGENCY CODE: CRISP
Yeast artificial chromosome clones and libraries for chromosome 11
PRINCIPAL INVESTIGATOR: HOEKSTRA, MERL F
ADDRESS: THE SALK INSTITUTE PO BOX 85800 SAN DIEGO, CA 92186-5800
PERFORMING ORG.: SALK INSTITUTE FOR BIOLOGICAL STUDIES, SAN DIEGO, CALIFORNIA
SPONSORING ORG.: NATIONAL CENTER FOR HUMAN GENOME RESEARCH
FY : 93 TYPE OF AWARD: Supplement (Type 3)
SUMMARY: Screening and organizing a large collection of clones for those that contain specific sequences is central to many applications in molecular biology. Standard hybridization methods for E. coli, phage, and yeast are of wide spread utility but are not ideally suited for all applications. Although colony hybridization techniques have proven effective as a screening method for identifying YAC clones containing single-copy genes, methods to facilitate the systematic and rapid identification of overlapping contiguous clones containing human genomic sequences are required. To this end we will develop new yeast artificial chromosome cloning vectors that will allow the direct visual identification of contiguous sequences by a replica plating assay. The system involves the preparation of two YAC libraries in yeast strains of opposite mating types and fusion of these cells into diploids that contain contiguous YACs that can undergo intermolecular homologous meiotic recombination. To reduce the load of the YAC containing transformants that would be required for the mating screen, we will prepare the chromosome 11 specific library from FACS-sorted DNA preparations. In addition to developing a recombination method for identifying contiguous sequences, we propose to develop an E. coli-yeast transposon mutagenesis system to allow the manipulation of cloned mammalian DNA segments through DNA-mediated transformation. This approach will allow the rapid targeting of sequences such as cDNAs onto existing YACs for such purposes as mapping exons, determining gene orientation within a YAC, identifying homologues, identifying structural features like 5' ends, and the construction of restriction maps of large DNA segments.

9/7/28 (Item 1 from file: 357)
134805 DBA Accession No.: 92-07297 PATENT
Immunoglobulin transgene and non-human animal containing it - human immunoglobulin heterologous monoclonal antibody expression in transgenic mouse
PATENT ASSIGNEE: Genpharm-Int. 1992
PATENT NUMBER: WO 9203918 PATENT DATE: 920319 WPI ACCESSION NO.: 92-113962 (9214)
PRIORITY APPLIC. NO.: US 575962 APPLIC. DATE: 900831
NATIONAL APPLIC. NO.: WO 91US6185 APPLIC. DATE: 910828
LANGUAGE: English

ABSTRACT: The following are new: (1) an immunoglobulin (Ig) heavy (H) chain and an Ig light (L) chain transgene encoding at least one V, D, J and C region gene segment, each segment being from (an individual of) the same species and capable of undergoing gene rearrangement in vivo; (2) a transgenic non-human animal (I) (preferably a mouse) containing heterologous Ig H- and L-chain transgenes in the germline; (3) a non-human B-lymphocyte derived from a (I), capable of producing a heterologous antibody; (4) a hybridoma capable of producing a heterologous monoclonal antibody (MAb); (5) production of (I) wherein at least one of the endogenous gene loci has been disrupted; (6) production of a heterologous antibody in a (I); (7) a MAb produced in (6); (8) production of a synthetic Ig V segment repertoire; (9) the synthetic Ig V segment produced in (8); (10) an Ig H- and L-chain minilocus transgene construct formed by many DNA fragments; (11) a yeast artificial chromosome (YAC) containing an Ig gene insert; and (12) a plasmid vector (plasmid pGP1b-plasmid pGP1f) useful for cloning Ig DNA fragments. (172pp)

9/7/29 (Item 2 from file: 357)
132403 DBA Accession No.: 92-04895 PATENT
Homologous recombination screening of DNA gene bank - constructed in eukaryotic host; Saccharomyces cerevisiae yeast artificial chromosome useful for chromosome walking and mapping
PATENT ASSIGNEE: Transkaryotic-Ther. 1992
PATENT NUMBER: WO 9201069 PATENT DATE: 920123 WPI ACCESSION NO.: 92-056884 (9207)
PRIORITY APPLIC. NO.: US 552183 APPLIC. DATE: 900713
NATIONAL APPLIC. NO.: WO 91US4926 APPLIC. DATE: 910712
LANGUAGE: English
ABSTRACT: A homologous-recombination method (I) for identifying and isolating target DNA in a gene bank in a eukaryotic host is claimed. (I) comprises: (a) providing a DNA gene bank in eukaryotic cells in which genetic recombination between DNA introduced into the host cells and DNA present in the host cells can occur; (b) introducing a targeting DNA vector plasmid YIp incapable of replicating in eukaryotic host cells and containing a selectable marker gene and a targeting DNA sequence homologous to part of the target DNA; (c) allowing recombination; and (d) selecting transformants. The following are also claimed: (1) mammalian, human or plant DNA or genes isolated by (I); (2) Saccharomyces cerevisiae TD7-16d, IV-16d and MGD131-10 carrying a chromosomal deletion in at least 1 selectable marker; (3) plasmid p184DLARG and its functional equivalents; (4) a plasmid which is non-replicating in yeast and contains a yeast and a bacterium selectable marker gene, a bacterial replication origin, and a cloning site; (5) a method for fragmenting human genomic DNA prior to mapping involving digesting human DNA with ApaI, NsiI or ScaI. (80pp)

9/7/30 (Item 1 from file: 434)
12440146 Genuine Article#: LL741 Number of References: 34
Title: A SERIES OF VECTORS THAT SIMPLIFY MAMMALIAN GENE TARGETING
Author(s): BROOKES AJ; STEVENSON BJ; PORTEOUS DJ; DORIN JR
Corporate Source: WESTERN GEN HOSP,MRC,HUMAN GENET UNIT,CREWE RD/EDINBURGH EH4 2XU/MIDLOTHIAN/SCOTLAND/
Journal: TRANSGENIC RESEARCH, 1993, V2, N4 (JUL), P238-244
ISSN: 0962-8819
Language: ENGLISH Document Type: NOTE
Abstract: In order to facilitate the procedure of mammalian gene targeting,

we have produced and functionally tested a series of generic vectors. Homologous recombination has been achieved with each vector. The vectors are designed for both replacement and insertional recombination, are suitable for 'hit and run' strategies and contain all necessary genetic elements for both positive-negative and promoterless/gene fusion enrichment of homologous integrations. Multiple unique restriction sites are included to simplify the incorporation of genomic targeting sequences.

9/7/31 (Item 2 from file: 434)
12389690 Genuine Article#: LG682 Number of References: 35
Title: 2 WNT GENES IN CAENORHABDITIS-ELEGANS
Author(s): SHACKLEFORD GM; SHIVAKUMAR S; SHIUE L; MASON J; KENYON C; VARMUS HE
Corporate Source: UNIV CALIF SAN FRANCISCO,DEPT MICROBIOL & IMMUNOL/SAN FRANCISCO//CA/94143; UNIV CALIF SAN FRANCISCO,DEPT MICROBIOL & IMMUNOL/SAN FRANCISCO//CA/94143; UNIV CALIF SAN FRANCISCO,DEPT ANAT/SAN FRANCISCO//CA/94143; UNIV CALIF SAN FRANCISCO,DEPT BIOCHEM & BIOPHYS/SAN FRANCISCO//CA/94143
Journal: ONCOGENE, 1993, V8, N7 (JUL), P1857-1864
ISSN: 0950-9232
Language: ENGLISH Document Type: ARTICLE
Abstract: wnt genes encode secretory glycoproteins that have been implicated in growth control and development in mice, frogs and insects. In this report we examine properties of two wnt genes recently identified in the nematode Caenorhabditis elegans. The first gene, Ce-wnt-1, was previously identified by a polymerase chain reaction-based screen of genomic DNA, and the second, Ce-wnt-2, was fortuitously encountered in a survey of clones in a cDNA library by the Caenorhabditis Genome Project. Full-length or nearly full-length cDNAs representing both mRNAs encode proteins that are similar in length, sequence and functional domains to other Wnt proteins. Primary products of 372 and 362 amino acids begin with a hydrophobic signal peptide, include two potential N-linked glycosylation sites and contain the 22 cysteine residues conserved throughout the wnt family. In contrast to mammalian and insect wnt genes with four or five exons and conserved intron-exon boundaries, Ce-wnt-1 has nine coding exons; only one of the eight identified introns interrupts the coding sequence at a position homologous to an intron position in other wnt genes. The major transcript derived from Ce-wnt-1 is 1.4 kb in length, and the 22 nucleotides at its 5' end are added by a trans-splicing mechanism. Ce-wnt-2 is also expressed via a single major transcript, 1.5 kb in length. Both RNAs are detectable in all larval forms and adults, but they are most abundant at the embryonic stage. Ce-wnt-1 is localized to the left arm of chromosome II and Ce-wnt-2 maps to a cluster of genes on chromosome IV.

9/7/32 (Item 3 from file: 434)
12329698 Genuine Article#: LC720 Number of References: 24
Title: AN INTEGRATED APPROACH FOR IDENTIFYING AND MAPPING HUMAN GENES
Author(s): DASGUPTA R; MORROW B; MARONDEL I; PARIMOO S; GOEI VL; GRUEN J; WEISSMAN S; SKOULTCHI A; KUCHERLAPATI R
Corporate Source: YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT MOLEC GENET/BRONX//NY/10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT MOLEC GENET/BRONX//NY/10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED,DEPT CELLBIOL/BRONX//NY/10461; YALE UNIV,SCH MED,BOYER CTR MOLEC MED/NEW HAVEN//CT/06536; YALE UNIV,SCH MED,DEPT PEDIAT/NEW HAVEN//CT/06536

Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1993, V90, N10 (MAY 15), P4364-4368
ISSN: 0027-8424
Language: ENGLISH Document Type: ARTICLE
Abstract: We have developed a method for generating expressed-sequence maps of human chromosomes. The method involves several steps that begin with libraries of highly representative short cDNAs prepared by using random oligomers as primers. The cDNA inserts are amplified by PCR with flanking vector primers. Chromosomal region-specific cDNA packets are prepared by hybridization of the cDNA inserts to DNA derived from yeast artificial chromosomes (YACs) assigned to defined regions of human chromosomes. The cDNA packets are cloned into yeast chromosome fragmentation vectors and used for transformation of yeast bearing the *YAC* used for affinity purification. Sequences in the cDNAs undergo *homologous* *recombination* with the corresponding exons in the genomic DNA yielding a set of truncated YACs. Each unique truncation specifies the location of an exon in the YAC. Since all of the truncation events end with the same vector sequence, it is possible to rescue and sequence these ends to generate expressed sequence tags. The method couples rapid purification of region-specific cDNAs with precise mapping of their genes on YACs. Appropriately truncated YACs also provide easy access to gene regulatory sequences. We describe the feasibility of individual steps of the method using the factor IX (F9) gene as a model system and we present the mapping of several expressed sequences corresponding to a 330-kb YAC containing DNA from human chromosome 6p21. In addition, we obtained the sequence, including an intron-exon junction, flanking a particular truncation event.

9/7/33 (Item 4 from file: 434)
11958935 Genuine Article#: JZ832 Number of References: 54
Title: THE CCR4 PROTEIN FROM SACCHAROMYCES-CEREVISIAE CONTAINS A LEUCINE-RICH REPEAT REGION WHICH IS REQUIRED FOR ITS CONTROL OF ADH2 GENE-EXPRESSION
Author(s): MALVAR T; BIRON RW; KABACK DB; DENIS CL
Corporate Source: UNIV NEW HAMPSHIRE,DEPT BIOCHEM & MOLEC BIOL/DURHAM//NH/03824; UMDNJ,NEW JERSEY MED SCH,DEPT MICROBIOL & MOLECGENET/NEWARK//NJ/07103
Journal: GENETICS, 1992, V132, N4 (DEC), P951-962
ISSN: 0016-6731
Language: ENGLISH Document Type: ARTICLE
Abstract: The CCR4 gene from Saccharomyces cerevisiae is required for the transcription of the glucose-repressible alcohol dehydrogenase (ADH2). Mutations in CCR4 also suppress the transcription at the ADH2 and his4-912delta loci caused by defects in the SPT10 (CRE1) and SPT6 (CRE2) genes. The CCR4 gene was mapped to the left arm of chromosome I and cloned by complementation of function using previously isolated segments of chromosome I. DNA sequence analysis of the cloned gene defined CCR4 as a 2511 bp open reading frame that would encode a polypeptide of 837 amino acids. The CCR4 mRNA was found to be 2.8 kb in size and Western analysis identified CCR4 as a 95,000 D protein. Disruption of the CCR4 gene resulted in reduced levels of ADH2 expression under both glucose and ethanol growth conditions and in temperature sensitive growth on nonfermentative medium, phenotypes essentially indistinguishable from previously identified mutations in CCR4. The amino terminus of the CCR4 protein was found to be rich in glutamine residues similar to a number of genes which are required for transcription. More importantly, CCR4 showed similarity to a diverse

set of proteins sharing a leucine-rich tandem repeat motif, the presence of which has been implicated in mediating protein-protein interactions. Deletions of several of the five leucine-rich repeats in CCR4 were shown to produce nonfunctional proteins indicating the importance of the repeats to CCR4 activity. This leucine-rich repeat region may mediate the contact CCR4 makes with another factor.

9/7/34 (Item 5 from file: 434)
11862096 Genuine Article#: JR858 Number of References: 54
Title: LOCALIZED MUTAGENESIS AND EVIDENCE FOR POSTTRANSCRIPTIONAL REGULATION OF MAK3 - A PUTATIVE N-ACETYLTRANSFERASE REQUIRED FOR DOUBLE-STRANDED-RNA VIRUS PROPAGATION IN SACCHAROMYCES-CEREVISIAE
Author(s): TERCERO JC; RILES LE; WICKNER RB
Corporate Source: NIDDKD,GENET SIMPLE EUKARYOTES SECT,BIOCHEM PHARMACOL LAB/BETHESDA//MD/20892; WASHINGTON UNIV,SCH MED,DEPT GENET/ST LOUIS//MO/63110
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1992, V267, N28 (OCT 5), P 20270-20276
ISSN: 0021-9258
Language: ENGLISH Document Type: ARTICLE
Abstract: The MAK3 gene of Saccharomyces cerevisiae is necessary for the propagation of the L-A double-stranded RNA virus and its satellites, such as M1 that encodes a killer toxin. We cloned the MAK3 gene based on its genetic map position using physically mapped lambda-clones covering nearly all of the yeast genome. The minimal sequence necessary to complement the mak3-1 mutation contained 3 open reading frames (ORFs). Only one (ORF3) was necessary to complement mak3-1. A deletion insertion mutant of ORF3 grew slowly on nonfermentable carbon sources, an effect not due simply to its loss of L-A. Although ORF3 alone is sufficient for MAK3 activity when expressed from an expression vector, in its native context an additional 669 base pairs 3' to the ORF and complementary to the gene for a non-histone protein are necessary for expression, but not for normal steady state transcript levels. This suggests a posttranscriptional control of MAK3 expression by the 3' region. The MAK3 protein has substantial homology with several N-acetyltransferases with consensus patterns h..h.h...Y..[HK]GI[AG][KR].Lh...h and h.h[DE] ....N..A...Y...GF.....Y...[DE]G, (h = hydrophobic). Mutation of any of the underlined conserved residues (94GI --> AA, 123N --> A, 130Y --> A, 134GF --> SL, 144Y --> A, and 149G --> A) inactivated the gene, supporting the hypothesis that MAK3 encodes an N-acetyltransferase.

9/7/35 (Item 6 from file: 434)
11799161 Genuine Article#: JM386 Number of References: 69
Title: A MUTANT NUCLEAR-PROTEIN WITH SIMILARITY TO RNA-BINDING PROTEINS INTERFERES WITH NUCLEAR IMPORT IN YEAST
Author(s): BOSSIE MA; DEHORATIUS C; BARCELO G; SILVER P
Corporate Source: PRINCETON UNIV,DEPT MOLEC BIOL/PRINCETON//NJ/08544
Journal: MOLECULAR BIOLOGY OF THE CELL, 1992, V3, N8 (AUG), P875-893
ISSN: 1059-1524
Language: ENGLISH Document Type: ARTICLE
Abstract: We have isolated mutants of the yeast Saccharomyces cerevisiae that are defective in localization of nuclear proteins. Chimeric proteins containing the nuclear localization sequence from SV40 large T-antigen fused to the N-terminus of the mitochondrial F1-beta-ATPase are localized to the nucleus. Npl (nuclear protein localization) mutants were isolated by their ability to grow on glycerol as a

consequence of no longer exclusively targeting SV40-F1-beta-ATPase to the nucleus. All mutants with defects in localization of nucleolar proteins and histones are temperature sensitive for growth at 36-degrees-C. Seven alleles of NPL3 and single alleles of several additional genes were isolated. NPL3 mutants were studied in detail. NPL3 encodes a nuclear protein with an RNA recognition motif and similarities to a family of proteins involved in RNA metabolism. Our genetic analysis indicates that NPL3 is essential for normal cell growth; cells lacking NPL3 are temperature sensitive for growth but do not exhibit a defect in localization of nuclear proteins. Taken together, these results indicate that the mutant forms of Npl3 protein isolated by this procedure are interfering with nuclear protein uptake in a general manner.

9/7/36 (Item 7 from file: 434)
11790461 Genuine Article#: JL614 Number of References: 37
Title: ELEVATED SISTER CHROMATID EXCHANGE PHENOTYPE OF BLOOM SYNDROME CELLS IS COMPLEMENTED BY HUMAN CHROMOSOME-15
Author(s): MCDANIEL LD; SCHULTZ RA
Corporate Source: UNIV MARYLAND,DIV HUMAN GENET/BALTIMORE//MD/21201; UNIV MARYLAND,CTR MED BIOTECHNOL/BALTIMORE//MD/21201
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1992, V89, N17 (SEP 1), P7968-7972
ISSN: 0027-8424
Language: ENGLISH Document Type: ARTICLE
Abstract: Bloom syndrome (BSx) is a rare autosomal-recessive chromosome-instability disorder manifested by a constellation of clinical features including a significant predisposition to early onset of neoplasia. BSx cells display cytogenetic abnormalities, the pathognomonic feature being an increased rate of spontaneous sister chromatid exchanges (SCEs), 10- to 15-fold more frequent than SCEs seen in control cells. Identification of the primary biochemical defect in BSx and its relationship to SCE frequency and neoplasia have been complicated by reports that BSx cell lines exhibit defects in the structure and/or activity of a number of different enzymes. The rare occurrence of the disorder and lack of informative families have precluded mapping of the primary defect by standard linkage analysis. We have utilized BSx cells as recipients for microcell-mediated chromosome transfer to map a locus that renders complementation of the elevated SCE phenotype. Studies with the BSx cell line GM08505 demonstrated a stable frequency of SCEs 10-fold higher than control values, offering a phenotype suitable for complementation studies. Transfer of different independent human chromosomes from somatic cell hybrids into BSx cells permitted identification of a single chromosome that dramatically reduced the SCE frequency to a level near that seen in control cells. Detailed characterization revealed this complementing element to be human chromosome 15.

9/7/37 (Item 8 from file: 434)
11781734 Genuine Article#: JK869 Number of References: 44
Title: ANALYSIS OF A GENE CONVERSION GRADIENT AT THE HIS4 LOCUS IN SACCHAROMYCES-CEREVISIAE
Author(s): DETLOFF P; WHITE MA; PETES TD
Corporate Source: UNIV N CAROLINA,DEPT BIOL/CHAPEL HILL//NC/27599; UNIV CHICAGO,DEPT MOLEC GENET & CELL BIOL/CHICAGO//IL/60637
Journal: GENETICS, 1992, V132, N1 (SEP), P113-123
ISSN: 0016-6731

Language: ENGLISH Document Type: ARTICLE
Abstract: Heteroduplexes formed between genes on homologous chromosomes are intermediates in meiotic recombination. In the HIS4 gene of Saccharomyces cerevisiae, most mutant alleles at the 5' end of the gene have a higher rate of meiotic recombination (gene conversion) than mutant alleles at the 3' end of the gene. Such gradients are usually interpreted as indicating a higher frequency of heteroduplex formation at the high conversion end of the gene. We present evidence indicating that the gradient of conversion at HIS4 primarily reflects the direction of mismatch repair rather than the frequency of heteroduplex formation. We also identify a site located between the 5' end of HIS4 and the 3' end of BIK1 that stimulates heteroduplex formation at HIS4 and BIK1.

9/7/38 (Item 9 from file: 434)
11781732 Genuine Article#: JK869 Number of References: 70
Title: MAK10, A GLUCOSE-REPRESSIBLE GENE NECESSARY FOR REPLICATION OF A DSRNA VIRUS OF SACCHAROMYCES-CEREVISIAE, HAS T-CELL RECEPTOR ALPHA SUBUNIT MOTIFS
Author(s): LEE YJ; WICKNER RB
Corporate Source: NIDDKD,BIOCHEM PHARMACOL LAB,GENET SIMPLE EUKARYOTES SECT/BETHESDA//MD/20892
Journal: GENETICS, 1992, V132, N1 (SEP), P87-96
ISSN: 0016-6731
Language: ENGLISH Document Type: ARTICLE
Abstract: The MAK10 gene is necessary for the propagation of the L-A dsRNA virus of the yeast Saccharomyces cerevisiae. We have isolated MAK10 from selected phage-gamma-genomic DNA clones that map near MAK10. This gene encodes a 733-amino acid protein with several regions of similarity to T cell receptor alpha-subunit V (variable) regions. We show that MAK10 is essential for optimal growth on nonfermentable carbon sources independent of its effect on L-A. Although loss of L-A by mak10-1 mutants is partially suppressed by loss of the mitochondrial genome, no such suppression of a mak10=URA3 mutation was observed. Using MAK10-lacZ fusions we show that MAK10 is expressed at a very low level and that it is glucose repressed. The highest levels of expression were seen in tup1 and cyc8 mutants, known to be defective in glucose repression. These results suggest that the mitochondrial genome and L-A dsRNA compete for the MAK10 protein.

9/7/39 (Item 10 from file: 434)
11778135 Genuine Article#: JJ802 Number of References: 36
Title: A SHUTTLE VECTOR WHICH FACILITATES THE EXPRESSION OF TRANSFECTED GENES IN TRYPANOSOMA-CRUZI AND LEISHMANIA
Author(s): KELLY JM; WARD HM; MILES MA; KENDALL G
Corporate Source: UNIV LONDON LONDON SCH HYG & TROP MED,DEPT MED PARASITOL,KEPPEL ST/LONDON WC1E 7HT//ENGLAND/
Journal: NUCLEIC ACIDS RESEARCH, 1992, V20, N15 (AUG 11), P3963-3969
ISSN: 0305-1048
Language: ENGLISH Document Type: ARTICLE
Abstract: A Trypanosoma cruzi expression vector has been constructed using sequences derived from the flanking regions of the glyceraldehyde 3-phosphate dehydrogenase (gGAPDH) genes. The neomycin phosphotransferase (neo(r)) gene was incorporated as a selectable marker. Using electroporation we have introduced this vector into both T.cruzi and Leishmania cells and conferred G418 resistance. Transformation is mediated by large extrachromosomal circular elements

composed of head-to-tail tandem repeats of the vector. The transformed phenotype is stable for at least 6 months in the absence of G418 and can be maintained during passage through the T.cruzi life-cycle. Foreign genes inserted into an expression site within the vector (pTEX) can be expressed at high levels in transformed cells. To our knowledge this paper describes the first trypanosome shuttle vector and the first vector which functions in both trypanosomes and Leishmania.

9/7/40 (Item 11 from file: 434)
11741622 Genuine Article#: JH148 Number of References: 53
Title: MEPS PARAMETERS AND GRAPH ANALYSIS FOR THE USE OF RECOMBINATION TO CONSTRUCT ORDERED SETS OF OVERLAPPING CLONES
Author(s): THALER DS; NOORDEWIER MO
Corporate Source: ROCKEFELLER UNIV,MOLEC GENET & INFORMAT LAB/NEWYORK//NY/10021; RUTGERS STATE UNIV,DEPT COMP SCI/NEW BRUNSWICK//NJ/08903
Journal: GENOMICS, 1992, V13, N4 (AUG), P1065-1074
Language: ENGLISH Document Type: ARTICLE

9/3/41 (Item 12 from file: 434)
11737331 Genuine Article#: JG912 No. References: 16
Title: CLONING OF CHROMOSOME-I DNA FROM SACCHAROMYCES-CEREVISIAE - ANALYSIS OF THE FUN52-GENE, WHOSE PRODUCT HAS HOMOLOGY TO PROTEIN-KINASES
Author(s): BARTON AB; DAVIES CJ; HUTCHISON CA; KABACK DB
Corporate Source: UNIV MED & DENT NEW JERSEY,NEW JERSEY MED SCH,GRAD SCH BIOMED SCI,DEPT MICROBIOL/NEWARK//NJ/07103; UNIV MED & DENT NEW JERSEY,NEW JERSEY MED SCH,GRAD SCH BIOMED SCI,DEPT MICROBIOL/NEWARK//NJ/07103; UNIV N CAROLINA,DEPT MICROBIOL & IMMUNOL/CHAPELHILL//NC/27599
Journal: GENE, 1992, V117, N1 (AUG 1), P137-140
Language: ENGLISH Document Type: NOTE (Abstract Available)

9/3/42 (Item 13 from file: 434)
11708504 Genuine Article#: JF088 No. References: 39
Title: COORDINATE REGULATION OF GLYCOGEN-METABOLISM IN THE YEAST SACCHAROMYCES-CEREVISIAE - INDUCTION OF GLYCOGEN BRANCHING ENZYME
Author(s): THON VJ; VIGNERONLESENS C; MARIANNEPEPIN T; MONTREUIL J; DECQ A; RACHEZ C; BALL SG; CANNON JF
Corporate Source: UNIV MISSOURI,SCH MED M642,DEPT MOLEC MICROBIOL& IMMUNOL/COLUMBIA//MO/65212; UNIV MISSOURI,SCH MED M642,DEPT MOLEC MICROBIOL& IMMUNOL/COLUMBIA//MO/65212; UNIV SCI & TECH LILLE FLANDRES ARTOIS,CHIM BIOLLAB,CNRS,UNITE MIXTE RECH 111/F-59655 VILLENEUVE DASCQ//FRANCE/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1992, V267, N21 (JUL 25), P 15224-15228
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/43 (Item 14 from file: 434)
11671830 Genuine Article#: JC128 No. References: 57
Title: EXPRESSION AND SECRETION OF PEA-SEED LIPOXYGENASE ISOENZYMES IN SACCHAROMYCES-CEREVISIAE
Author(s): KNUST B; VONWETTSTEIN D
Corporate Source: CARLSBERG LAB,DEPT PHYSIOL,GAMLE CARLSBERG VEJ 10/DK-2500 COPENHAGEN//DENMARK/; DANISCO AS,RES LAB/COPENHAGEN S//DENMARK/
Journal: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, 1992, V37, N3 (JUN), P 342-351
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/44 (Item 15 from file: 434)
11652062 Genuine Article#: JA763 No. References: 49
Title: KINESIN-RELATED PROTEINS REQUIRED FOR ASSEMBLY OF THE MITOTIC SPINDLE
Author(s): ROOF DM; MELUH PB; ROSE MD
Corporate Source: PRINCETON UNIV,DEPT MOLEC BIOL/PRINCETON//NJ/08544
Journal: JOURNAL OF CELL BIOLOGY, 1992, V118, N1 (JUL), P95-108
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/45 (Item 16 from file: 434)
11642867 Genuine Article#: HZ673 No. References: 50
Title: A RIBOSOMAL-RNA GENE PROMOTER AT THE TELOMERE OF A MINICHROMOSOME IN TRYPANOSOMA-BRUCEI
Author(s): ZOMERDIJK JCBM; KIEFT R; BORST P
Corporate Source: NETHERLANDS CANC INST,DIV MOLEC BIOL,PLESMANLAAN 121/1066 CX AMSTERDAM//NETHERLANDS/; NETHERLANDS CANC INST,DIV MOLEC BIOL,PLESMANLAAN 121/1066 CX AMSTERDAM//NETHERLANDS/
Journal: NUCLEIC ACIDS RESEARCH, 1992, V20, N11 (JUN 11), P2725-2734
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/46 (Item 17 from file: 434)
11642862 Genuine Article#: HZ673 No. References: 39
Title: TARGETED ALTERATIONS IN YEAST ARTIFICIAL CHROMOSOMES FOR INTERSPECIES GENE-TRANSFER
Author(s): DAVIES NP; ROSEWELL IR; BRUGGEMANN M
Corporate Source: AFRC,INST ANIM PHYSIOL & GENET RES/CAMBRIDGE CB2 4AT//ENGLAND/; AFRC,INST ANIM PHYSIOL & GENET RES/CAMBRIDGE CB2 4AT//ENGLAND/
Journal: NUCLEIC ACIDS RESEARCH, 1992, V20, N11 (JUN 11), P2693-2698
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/47 (Item 18 from file: 434)
11621610 Genuine Article#: HY053 No. References: 20
Title: MEIOTIC RECOMBINATION AND SEGREGATION OF HUMAN-DERIVED ARTIFICIAL CHROMOSOMES IN SACCHAROMYCES-CEREVISIAE
Author(s): SEARS DD; HEGEMANN JH; HIETER P
Corporate Source: JOHNS HOPKINS UNIV,SCH MED/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED/BALTIMORE//MD/21205; UNIV GIESSEN,INST MIKROBIOL & MOLEK BIOL/W-6300GIESSEN//GERMANY/
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1992, V89, N12 (JUN 15), P5296-5300
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/48 (Item 19 from file: 434)
11612085 Genuine Article#: HX298 No. References: 22
Title: ANALYSIS OF THE CHROMOSOMAL DNA POLYMORPHISM OF WINE STRAINS OF SACCHAROMYCES-CEREVISIAE
Author(s): BIDENNE C; BLONDIN B; DEQUIN S; VEZINHET F
Corporate Source: INRA,INST PROD VIGNE,MICROBIOL & TECHNOL FERMENTAT LAB,2 PL VIALA/F-34060 MONTPELLIER 1//FRANCE/; INRA,INST PROD VIGNE,MICROBIOL & TECHNOL FERMENTAT LAB,2 PL VIALA/F-34060 MONTPELLIER 1//FRANCE/; ECOLE NATL SUPER AGRON,INST PROD VIGNE,CHAIRE TECHNOL ALIMENTAIRE & OENOL/F-34069 MONTPELLIER 1//FRANCE/
Journal: CURRENT GENETICS, 1992, V22, N1 (JUL), P1-7
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/49 (Item 20 from file: 434)
11597251 Genuine Article#: HV961 No. References: 39
Title: YML9, A NUCLEUS-ENCODED MITOCHONDRIAL RIBOSOMAL-PROTEIN OF YEAST, IS HOMOLOGOUS TO L3 RIBOSOMAL-PROTEINS FROM ALL NATURAL KINGDOMS AND PHOTOSYNTHETIC ORGANELLES
Author(s): GRAACK HR; GROHMANN L; KITAKAWA M; SCHAFER KL; KRUFT V
Corporate Source: UNIV HAMBURG,KRANKENHAUS EPPENDORF,ZENTRUM MOLEK NEUROBIOL,PONGS ABT,MARTINISTR 52/W-2000 HAMBURG 20//GERMANY/; MAX PLANCK INST MOLEC GENET,WITTMANN ABT/W-1000BERLIN 33//GERMANY/; RUHR UNIV BOCHUM,FAK CHEM,LEHRSTUHL BIOCHEM/W-4630 BOCHUM//GERMANY/
Journal: EUROPEAN JOURNAL OF BIOCHEMISTRY, 1992, V206, N2 (JUN 1), P373-380
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/50 (Item 21 from file: 434)
11586274 Genuine Article#: HU938 No. References: 45
Title: CLONING AND CHARACTERIZATION OF THE CYS3 (CYI1) GENE OF SACCHAROMYCES-CEREVISIAE
Author(s): ONO BI; TANAKA K; NAITO K; HEIKE C; SHINODA S; YAMAMOTO S; OHMORI S; OSHIMA T; TOHE A
Corporate Source: OKAYAMA UNIV,FAC PHARMACEUT SCI,ENVIRONM HYG CHEM LAB/OKAYAMA 700//JAPAN/; OKAYAMA UNIV,FAC PHARMACEUT SCI,PHYSIOL CHEM LAB/OKAYAMA 700//JAPAN/; UNIV TOKYO,DEPT BOT/TOKYO 113//JAPAN/; SUNTORY BIOPHARMA TECH CTR/TOKYO/GUNMA 37005/JAPAN/
Journal: JOURNAL OF BACTERIOLOGY, 1992, V174, N10 (MAY), P3339-3347
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/51 (Item 22 from file: 434)
11564959 Genuine Article#: HT777 No. References: 44
Title: GENETIC AND MOLECULAR ANALYSIS OF DNA43 AND DNA52 - 2 NEW CELL-CYCLE GENES IN SACCHAROMYCES-CEREVISIAE
Author(s): SOLOMON NA; WRIGHT MB; CHANG S; BUCKLEY AM; DUMAS LB; GABER RF
Corporate Source: NORTHWESTERN UNIV,DEPT BIOCHEM MOLEC BIOL & CELL BIOL/EVANSTON//IL/60208; NORTHWESTERN UNIV,DEPT BIOCHEM MOLEC BIOL & CELL BIOL/EVANSTON//IL/60208
Journal: YEAST, 1992, V8, N4 (APR), P273-289
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/52 (Item 23 from file: 434)
11518145 Genuine Article#: HN934 No. References: 25
Title: CLONING AND MAPPING OF THE CYS4 GENE OF SACCHAROMYCES-CEREVISIAE
Author(s): ONO B; HEIKE C; YANO Y; INOUE T; NAITO K; NAKAGAMI S; YAMANE A
Corporate Source: OKAYAMA UNIV,FAC PHARMACEUT SCI,ENVIRONM HYG CHEM LAB/OKAYAMA 700//JAPAN/; WAKUNAGA PHARMACEUT CO LTD,INST BIOTECHNOL RES/KODA/HIROSHIMA 72964/JAPAN/
Journal: CURRENT GENETICS, 1992, V21, N4-5 (APR), P285-289
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/53 (Item 24 from file: 434)
11431164 Genuine Article#: HH195 No. References: 34
Title: GENE ISOLATION BY COMPLEMENTATION IN CANDIDA-ALBICANS AND APPLICATIONS TO PHYSICAL AND GENETIC-MAPPING
Author(s): GOSHORN AK; GRINDLE SM; SCHERER S
Corporate Source: UNIV MINNESOTA,SCH MED,DEPT MICROBIOL,BOX 196 UMHC/MINNEAPOLIS//MN/55455; UNIV MINNESOTA,SCH MED,DEPT MICROBIOL,BOX 196 UMHC/MINNEAPOLIS//MN/55455
Journal: INFECTION AND IMMUNITY, 1992, V60, N3 (MAR), P876-884
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/54 (Item 25 from file: 434)
11407128 Genuine Article#: HG601 No. References: 40
Title: IDENTIFICATION OF CRYPTIC LOX SITES IN THE YEAST GENOME BY SELECTION FOR CRE-MEDIATED CHROMOSOME TRANSLOCATIONS THAT CONFER MULTIPLE-DRUG RESISTANCE
Author(s): SAUER B
Corporate Source: DUPONT MERCK PHARMACEUT CO,EXPTL STN E328/WILMINGTON//DE/19880
Journal: JOURNAL OF MOLECULAR BIOLOGY, 1992, V223, N4 (FEB 20), P911-928
Language: ENGLISH Document Type: ARTICLE

9/3/55 (Item 26 from file: 434)
11384457 Genuine Article#: HE838 No. References: 39
Title: MOLECULAR AND GENETIC-ANALYSIS OF THE YEAST EARLY MEIOTIC RECOMBINATION GENES REC102 AND REC107/MER2
Author(s): COOL M; MALONE RE
Corporate Source: UNIV IOWA,DEPT BIOL/IOWA CITY//IA/52242; UNIV IOWA,DEPT BIOL/IOWA CITY//IA/52242
Journal: MOLECULAR AND CELLULAR BIOLOGY, 1992, V12, N3 (MAR), P1248-1256
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/56 (Item 27 from file: 434)
11370663 Genuine Article#: HD702 No. References: 36
Title: THE COMPLETE SEQUENCE OF A 10.8KB FRAGMENT TO THE RIGHT OF THE CHROMOSOME-III CENTROMERE OF SACCHAROMYCES-CEREVISIAE
Author(s): BITEAU N; FREMAUX C; HEBRARD S; MENARA A; AIGLE M; CROUZET M
Corporate Source: UNIV BORDEAUX 2,BIOL MOLEC & SEQUENCAGE LAB,146RUE LEO SAIGNAT/F-33076 BORDEAUX//FRANCE/; UNIV BORDEAUX 2,BIOL MOLEC & SEQUENCAGE LAB,146RUE LEO SAIGNAT/F-33076 BORDEAUX//FRANCE/
Journal: YEAST, 1992, V8, N1 (JAN), P61-70
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/57 (Item 28 from file: 434)
11293709 Genuine Article#: GY629 No. References: 31
Title: NEW YEAST ACTIN-LIKE GENE REQUIRED LATE IN THE CELL-CYCLE
Author(s): SCHWOB E; MARTIN RP
Corporate Source: CNRS,INST BIOL MOLEC & CELLULAIRE,BIOCHIM LAB,15 RUE RENE DESCARTES/F-67084 STRASBOURG//FRANCE/; CNRS,INST BIOL MOLEC & CELLULAIRE,BIOCHIM LAB,15 RUE RENE DESCARTES/F-67084 STRASBOURG//FRANCE/; UNIV STRASBOURG 1/F-67084 STRASBOURG//FRANCE/
Journal: NATURE, 1992, V355, N6356 (JAN 9), P179-182
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/58 (Item 29 from file: 434)
11286752 Genuine Article#: GX543 No. References: 48
Title: THE REC102 MUTANT OF YEAST IS DEFECTIVE IN MEIOTIC RECOMBINATION AND CHROMOSOME SYNAPSIS
Author(s): BHARGAVA J; ENGEBRECHT J; ROEDER GS
Corporate Source: YALE UNIV,DEPT BIOL/NEW HAVEN//CT/06511
Journal: GENETICS, 1992, V130, N1 (JAN), P59-69
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/59 (Item 30 from file: 434)
11274594 Genuine Article#: GX544 No. References: 65
Title: A MEIOSIS-SPECIFIC PROTEIN-KINASE HOMOLOG REQUIRED FOR CHROMOSOME SYNAPSIS AND RECOMBINATION

Author(s): ROCKMILL B; ROEDER GS
Corporate Source: YALE UNIV,DEPT BIOL/NEW HAVEN//CT/06511
Journal: GENES & DEVELOPMENT, 1991, V5, N12B (DEC), P2392-2404
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/60 (Item 31 from file: 434)
11267111 Genuine Article#: GW195 No. References: 13
Title: A NEW-PROTEIN KINASE, SSP31, MODULATING THE SMP3 GENE-PRODUCT INVOLVED IN PLASMID MAINTENANCE IN SACCHAROMYCES-CEREVISIAE
Author(s): IRIE K; ARAKI H; OSHIMA Y
Corporate Source: OSAKA UNIV,FAC ENGN,DEPT BIOTECHNOL,2-1 YAMADAOKA/SUITA/OSAKA 565/JAPAN/; OSAKA UNIV,FAC ENGN,DEPT BIOTECHNOL,2-1 YAMADAOKA/SUITA/OSAKA 565/JAPAN/
Journal: GENE, 1991, V108, N1 (DEC 1), P139-144
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

9/3/61 (Item 32 from file: 434)
11234968 Genuine Article#: GU008 No. References: 28
Title: DIRECT CLONING OF A LONG RESTRICTION FRAGMENT AIDED WITH A JUMPING CLONE
Author(s): MATSUOKA T; KATO H; HASHIMOTO K; KUROSAWA Y
Corporate Source: FUJITA HLTH UNIV,INST COMPREHENS MED SCI/TOYOAKE/AICHI 47011/JAPAN/; FUJITA HLTH UNIV,INST COMPREHENS MED SCI/TOYOAKE/AICHI 47011/JAPAN/
Journal: GENE, 1991, V107, N1, P27-35
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

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=> s (yac# or yeast(w)artificial(w)chromosom?)/ab,bi
           885 YAC#/AB
           259 YAC#/BI
         54274 YEAST/AB
         43428 YEAST/BI
         27220 ARTIFICIAL/AB
         17577 ARTIFICIAL/BI
         47669 CHROMOSOM?/AB
         33285 CHROMOSOM?/BI
           373 YEAST(W)ARTIFICIAL(W)CHROMOSOM?
L1         997 (YAC# OR YEAST(W)ARTIFICIAL(W)CHROMOSOM?)/AB,BI

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          4166 TRANSGEN?/BI
L2        5548 TRANSGEN?/AB,BI

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L3          11 L1 (L) L2

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L3   ANSWER 1 OF 11  COPYRIGHT 1993 ACS
AN   CA119(5):42155k
TI   Transgenic mice containing a human heavy chain
     immunoglobulin gene fragment cloned in a yeast
   artificial chromosome
SO   Nat. Genet., 4(2), 117-23
AU   Choi, Ted K.; Hollenbach, Paul W.; Pearson, Barbara E.; Ueda, Roanna
     M.; Weddell, Gregory N.; Kurahara, Carole G.; Woodhouse, Clive S.;
     Kay, Robert M.; Loring, Jeanne F.
PY   1993
AN   CA119(5):42155k
AB   The authors developed a method for the introduction of yeast
   artificial chromosomes (YACs) into
   transgenic mice. An 85 kilobase (kb) fragment of the human
     heavy chain immunoglobulin gene was cloned as a YAC, and
     embryonic stem cell lines carrying intact, integrated YACS
     were derived by co-lipofection of the YAC with an unlinked
     selectable marker. Chimeric founder animals were produced by
     blastocyst injection, and offspring transgenic for the
   YAC were obtained. Anal. of serum from these offspring for
     human heavy chain antibody subunits demonstrated expression of the
   YAC-borne immunoglobulin gene fragment. Co-lipofection may
     prove to be a highly-successful means of producing
   transgenic mice contg. large gene fragments in YACs
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L3 ANSWER 2 OF 11 COPYRIGHT 1993 ACS
AN CA119(1):1935w
TI A **yeast artificial chromosome** covering the tyrosinase gene confers copy number-dependent expression in **transgenic** mice
SO Nature (London), 362(6417), 258-61
AU Schedl, Andreas; Montoliu, Lluis; Kelsey, Gavin; Schuetz, Guenther
PY 1993
AN CA119(1):1935w
AB A 250-kb **yeast artificial chromosome** (**YAC**) has been constructed contg. 80 kb of the mouse tyrosinase coding region, 155 kb of upstream sequences, and 15 kb of vector DNA. The **YAC**, designated YRT2, covering the mouse tyrosinase gene was transferred into mice by pronuclear injection of gel-purified **YAC** DNA. The **YAC** was inserted into the mouse genome without major rearrangements and expression of the **YAC**-borne tyrosinase gene resulted in complete rescue of the albino phenotype of the recipient mice. Expression from the **transgene** reached levels comparable to that of the endogenous gene and showed copy no. dependence and position independence.

L3 ANSWER 3 OF 11 COPYRIGHT 1993 ACS
AN CA118(23):226809n
TI Germ line transmission of a yeast artificial chromosome spanning the murine .alpha.1(I) collagen locus
SO Science (Washington, D. C., 1883-), 259(5103), 1904-7
AU Strauss, William M.; Dausman, Jessica; Beard, Caroline; Johnson, Carol; Lawrence, Jeanne B.; Jaenisch, Rudolf
PY 1993
AN CA118(23):226809n
AB Mol. complementation of mutant phenotypes by **transgenic** technol. is a potentially important tool for gene identification. A technol. was developed that allows the transfer of a phys. intact **yeast artificial chromosome** (**YAC**) into the germ line of the mouse. A purified 150-kilobase **YAC** encompassing the murine gene Col1a1 was efficiently introduced into embryonic stem (ES) cells via lipofection. Chimeric founder mice were derived from 2 transfected ES cell clones. These chimeras transmitted the full length **transgene** through the germ line, generating 2 **transgenic** mouse strains. **Transgene** expression was visualized as nascent transcripts in interphase nuclei and quantitated by RNase protection anal. Both assays indicated that the **transgene** was expressed at levels comparable to the endogenous collagen gene.

L3 ANSWER 4 OF 11 COPYRIGHT 1993 ACS
AN CA118(23):226752p
TI Rapid physical mapping of YAC inserts by random integration of I-Sce I sites
SO Hum. Mol. Genet., 2(3), 265-71
AU Colleaux, Laurence; Rougeulle, Claire; Avner, Philip; Dujon, Bernard
PY 1993
AN CA118(23):226752p
AB This report describes a novel strategy, based on the random

insertion by homologous recombination of artificial I-SceI sites within mammalian repetitive DNA sequences, which should greatly facilitate the high resoln. phys. mapping of large DNA fragments cloned in **YAC**. A set of **transgenic** yeast strains contg. appropriately spaced I-SceI sites within the **YAC** insert defines a series of nested phys. intervals against which new genes, clones or DNA fragments can be mapped by simple hybridization. Sequential hybridization using such a series of nested **YAC** fragments as probes can also allow the rapid sorting of phage or cosmid libraries into contigs. This approach, which has been applied to a **YAC** contg. a 460 kb insert from the mouse X chromosome, may also have applications for the restriction mapping of large genomic segments, mapping of exons and the search for homologous genes.

L3 ANSWER 5 OF 11 COPYRIGHT 1993 ACS
AN CA118(19):185125r
TI Introduction of very large DNA fragments into embryonic stem cells as yeast artificial chromosomes
SO PCT Int. Appl., 16 pp.
AU Bruggemann, Marianne
PI WO 9305165 A1 18 Mar 1993
AI WO 92-GB1651 10 Sep 1992
PY 1993
AN CA118(19):185125r
AB Very large pieces of cloned DNA (>100 kb) can be introduced into embryonic stem cells as yeast artificial chromosomes. This allows the introduction of complex genetic regions such as parts of the Ig-coding region; it also allows the introduction of a gene with all of its regulatory regions. The DNA may be introduced by std. methods such as microinjection and selectable markers and methods to stabilize the transforming DNA, e.g. forced integration, may be used.

L3 ANSWER 6 OF 11 COPYRIGHT 1993 ACS
AN CA118(5):33660y
TI Efficient joining of large DNA fragments for transgenesis
SO Nucleic Acids Res., 20(22), 6109-10
AU Strouboulis, John; Dillon, Niall; Grosveld, Frank
PY 1992
AN CA118(5):33660y
AB A novel method was devised to introduce large DNA fragments into the germline of **transgenic** mice. The method is based on in vitro ligation of complementary single stranded tails at the end of two overlapping cosmids from the human .beta. globin locus, resulting in a 70 kb fragment that was purified by gel electrophoresis. By microinjection at low DNA concn., **transgenic** mice were obtained that contain a single copy of the human .beta. globin locus. Southern blot data show that these single copy loci are intact and the junction unrearranged. The ability to reconstruct large fragments in vitro provides a rapid and flexible method to study the regulation of large complex loci in **transgenic** mice and avoids the complications inherent in the use of coinjection of large fragments. Coinjection gives complicated integration patterns, while generally **yeast artificial chromosome** technol. is less flexible for manipulation.

L3 ANSWER 7 OF 11 COPYRIGHT 1993 ACS
AN CA117(13):129851s
TI Transgenic non-human animals capable of producing heterologous antibodies
SO PCT Int. Appl., 166 pp.
AU Lonberg, Nils; Kay, Robert M.
PI WO 9203918 A1 19 Mar 1992
AI WO 91-US6185 28 Aug 1991
PY 1992
AN CA117(13):129851s
AB Heterologous antibodies are synthesized by transgenic mammals into which the transforming DNA is introduced at the zygote or embryo stage. Such animals are used for the manuf. of humanized antibodies (no data). The prepn. of such animals may be by multiple transformation of embryos with all the necessary expression units or by crossing of animals carrying genes for individual expression units. The expression units may contain unmodified coding regions or minigene coding regions. The cloning of the Ig coding regions (before or after somatic recombination) by std. methods and their use in the construction of expression plasmids is described. Deletion of corresponding regions of the mouse genome to prevent synthesis of the corresponding mouse Igs by homologous recombination is also described. Mouse embryos injected with human IgM genes had human IgM in their serum.

L3 ANSWER 8 OF 11 COPYRIGHT 1993 ACS
AN CA117(11):105378s
TI **Transgenic** mice generated by pronuclear injection of a **yeast artificial chromosome**
SO Nucleic Acids Res., 20(12), 3073-7
AU Schedl, Andreas; Beermann, Friedrich; Thies, Edda; Montoliu, Lluis; Kelsey, Gavin; Schuetz, Guenther
PY 1992
AN CA117(11):105378s
AB **Transgenic** mice have become invaluable for analyzing gene function and regulation in vivo. However, the size of constructs injected has been limited by the cloning capacity of conventional vectors, a constraint that could be overcome with **yeast artificial chromosomes** (**YACs**). The feasibility of making **transgenic** mice with **YACs** by pronuclear injection of a small **YAC** carrying a gene encoding tyrosinase was investigated. Use of a vector with a conditional centromere allowed 15-fold amplification of the **YAC** in yeast and its recovery in high yield. The albino phenotypes of the recipient mice were rescued demonstrating the correct expression of the tyrosinase gene from the construct. Furthermore, the telomeric sequences added by the yeast integrated into the mouse genome and did not reduce efficiency of integration. Using this technique future expts. with longer **YACs** will allow the expression of gene complexes such as Hox and the globin gene clusters to be analyzed in **transgenic** animals.

L3 ANSWER 9 OF 11 COPYRIGHT 1993 ACS
AN CA115(9):90543z
TI Alteration of the natural killer repertoire in H-2 transgenic mice: specificity of rapid lymphoma cell clearance determined by the H-2

phenotype of the target
SO J. Exp. Med., 174(2), 327-34
AU Hoeglund, Petter; Glas, Rickard; Oehlen, Claes; Ljunggren, Hans Gustaf; Kaerre, Klas
PY 1991
AN CA115(9):90543z
AB The mechanism behind natural tumor resistance conveyed by a H-2Dd **transgene** to C57Bl/6 (B6) mice was investigated. **Transgenic** D8 mice were more efficient than control mice in natural killer (NK) cell mediated rapid elimination of i.v. inoculated radiolabeled lymphoma cells of B6 origin, such as RBL-5. There was no difference between D8 and B6 mice when elimination of **YAC**-1 targets was monitored. The effect of the **transgene** on the NK repertoire was related to the H-2 phenotype of the target: the differential elimination of RBL-5 lymphoma cells in D8 and B6 mice was not seen when a H-2 deficient variant of this line was used (efficiently eliminated in both genotypes), nor was it seen with a H-2Dd transfectant (surviving in both genotypes). The data show that a MHC class I **transgene** can directly control natural killing in vivo by altering the repertoire rather than the general levels of NK activity. Since the NK mediated elimination seen after introduction of a novel gene in the host was neutralized by introducing the same gene (H-2Dd), but not an unrelated class I gene (H-2Dp), in the tumor, the data support the concept of NK surveillance against missing self. This combined **transgenic**/transfectant system may serve as a tool for a mol. dissection of the interactions between NK cells and their targets in vivo.

L3 ANSWER 10 OF 11 COPYRIGHT 1993 ACS
AN CA115(9):86773g
TI Yeast artificial chromosomes in diagnosis of inherited diseases such as cystic fibrosis
SO Eur. Pat. Appl., 36 pp.
AU Anand, Rakesh; Markham, Alexander Fred; Smith, John Craig; Anwar, Rashida; Riley, John Hamilton; Ogilvie, Donald James; Elvin, Paul
PI EP 416801 A2 13 Mar 1991
AI EP 90-309420 29 Aug 1990
PY 1991
AN CA115(9):86773g
AB A DNA hybridization method for diagnosis of inherited diseases such as cystic fibrosis is based on the presence or absence of a variant nucleotide sequence in specified **yeast artificial chromosomes (YACs)**. A method for the prepn. of a **transgenic** cell line or a **transgenic** animal contg. a **YAC** contg. a cystic fibrosis locus, and a kit comprising polynucleotide probes and/or primers for diagnosis of cystic fibrosis are also described.

L3 ANSWER 11 OF 11 COPYRIGHT 1993 ACS
AN CA113(11):92537h
TI A yeast artificial chromosome containing the mouse homeobox cluster Hox-2
SO Proc. Natl. Acad. Sci. U. S. A., 87(12), 4751-5
AU Rubock, Melissa J.; Larin, Zoia; Cook, Martyn; Papalopulu, Nancy; Krumlauf, Robb; Lehrach, Hans
PY 1990

AN CA113(11):92537h
AB Two genes, Hox-2.8 and Hox-2.9, from the mouse homeobox cluster Hox-2, located on chromosome 11 were isolated. A 120-kilobase **yeast artificial chromosome** (**YAC**) contg. a large region of the murine Hox-2 cluster, including 45 kilobases of sequence upstream of the most 5' gene, was cloned. The DNA sequence of the **YAC** is unrearranged relative to the genomic map. The authors subcloned from the **YAC** insert a homeobox gene, Hox-2.8, whose homeodomain is highly related to that of the Drosophila homeotic gene proboscopedia (pb). The expression pattern of Hox-2.8 during embryogenesis extends the trend established by genes from Hox-2.5 to -2.7 of successively anterior domains of expression in the neural tube. Also subcloned and sequenced from a cosmid was the labial (lab)-related Hox-2.9, the most 3' member of the cluster to date. These data lend further support to the idea of a common evolutionary origin of the mouse Hox and Drosophila HOM clusters. The **YAC** may be useful to construct modified forms of the Hox-2 cluster in yeast and to identify their effect on the phenotype of the animal in **transgenic** mouse strains.

=> s (l1 and l2) not l3
L4 1 (L1 AND L2) NOT L3

=> d .caabs

L4 ANSWER 1 OF 1 COPYRIGHT 1993 ACS
AN CA118(9):78665v
TI Inherited and somatic mutations of the APC gene associated with colorectal cancer of humans
SO PCT Int. Appl., 138 pp.
AU Kinzler, Kenneth W.; Vogelstein, Bert; Anand, Rakesh; Hedge, Philip John; Markham, Alexander Fred; Albertsen, Hans; Carlson, Mary L.; Groden, Joanna L.; Joslyn, Geoff; et al.
PI WO 9213103 A1 6 Aug 1992
AI WO 92-US376 16 Jan 1992
PY 1992
AN CA118(9):78665v
AB A human gene that shows inherited and somatic mutations assocd. with colorectal cancer is cloned and characterized. The gene and its product are useful as markers in the diagnosis and prognosis of the disease. A series of **YAC** clones of the 5q21 region were cloned by screening with markers for the region. Six genes expressed in normal colon cells and in colorectal, lung and bladder tumors were found in the region. These genes were: the FER gene at 5q11-23 similar to the v-abl gene; TB1 showing some similarity to brown adipose tissue uncoupling proteins; MCC and TB2; and APC. A cDNA from the APC gene had an open reading frame of 8,535 nucleotides that encoded a protein with some similarity to myosins and intermediate filament proteins and to to the ral2 gene product of yeast. The assocn. of these genes and mutant alleles with colorectal cancer was studied by std. methods. The gene that showed the greatest no. of germline and somatic mutations was APC and the characterization of a no. of the mutations is described.

=> s (l1 (l) (recombin? or homologo?)/ab,bi) not (l3 or l4)
81004 RECOMBIN?/AB

```
         48787 RECOMBIN?/BI
         33823 HOMOLOGO?/AB
          8176 HOMOLOGO?/BI
           167 L1 (L) (RECOMBIN? OR HOMOLOGO?)/AB,BI
L5         165 (L1 (L) (RECOMBIN? OR HOMOLOGO?)/AB,BI) NOT (L3 OR L4)


=> s (l1 (l) (recombin?/ab,bi)) not (l3 or l4)
         81004 RECOMBIN?/AB
         48787 RECOMBIN?/BI
           146 L1 (L) (RECOMBIN?/AB,BI)
L6         144 (L1 (L) (RECOMBIN?/AB,BI)) NOT (L3 OR L4)

=> s l5 and homologo?/ab,bi
         33823 HOMOLOGO?/AB
          8176 HOMOLOGO?/BI
L7          52 L5 AND HOMOLOGO?/AB,BI

=> d .caabs l7 1-52

L7   ANSWER 1 OF 52  COPYRIGHT 1993 ACS
AN   CA119(3):21612s
TI   Incorporation of copy-number control elements into yeast
   artificial chromosomes by targeted
   homologous recombination
SO   Mamm. Genome, 4(3), 141-7
AU   Smith, Douglas R.; Smyth, Adrienne P.; Strauss, William M.; Moir,
     Donald T.
PY   1993
AN   CA119(3):21612s
AB   A pair of vectors was developed for exchanging yeast
   artificial chromosome (YAC) arms by
     targeted homologous recombination. These
     conversion vectors allow the introduction of copy-no. control
     elements into YACs constructed with pYAC4 or related
     vectors. YACs modified in this way provide an enriched
     source of DNA for genetic or biochem. studies. A LYS2 gene on the
     conversion vector provides a genetic selection for the modified
   YACs after transformation with appropriately prepd. vector.
     A background of Lys+ clones that do not contain modified
   YACs is also present. However, clones with converted
   YACs can be distinguished from this background by
     counter-screening for loss of the original pYAC4 TRP1 arm (Trp-
     phenotype). The elimination of yeast replication origins (ARS
     elements) from the conversion vectors increased the frequency of
     Lys+ Trp- clones, but resulted in weaker amplification. Several
   YACs were converted with these vectors, and the fate of the
     transformed DNA and of the resident YAC DNA was
     systematically investigated.

L7   ANSWER 2 OF 52  COPYRIGHT 1993 ACS
AN   CA119(3):21549b
TI   An integrated approach for identifying and mapping human genes
SO   Proc. Natl. Acad. Sci. U. S. A., 90(10), 4364-8
AU   Das Gupta, Ruchira; Morrow, Bernice; Marondel, Ivonne; Parimoo,
     Satish; Goei, Vita L.; Gruen, Jeffrey; Weissman, Sherman; Skoultchi,
     Arthur; Kucherlapati, Raju
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PY 1993
AN CA119(3):21549b
AB A method was developed for generating expressed-sequence maps of human chromosomes. The method involves several steps that begin with libraries of highly representative short cDNAs prepd. by using random oligomers as primers. The cDNA inserts are amplified by PCR with flanking vector primers. Chromosomal region-specific cDNA packets are prepd. by hybridization of the cDNA inserts to DNA derived from **yeast artificial chromosomes** (**YACs**) assigned to defined regions of human chromosomes. The cDNA packets are cloned into yeast chromosome fragmentation vectors and used for transformation of yeast bearing the **YAC** used for affinity purifn. Sequences in the cDNAs undergo **homologous recombination** with the corresponding exons in the genomic DNA yielding a set of truncated **YACs**. Each unique truncation specifies the location of an exon in the **YAC**. Since all of the truncation events end with the same vector sequence, it is possible to rescue and sequence these ends to generate expressed sequence tags. The method couples rapid purifn. of region-specific cDNAs with precise mapping of their genes on **YACs**. Appropriately truncated **YACs** also provide easy access to gene regulatory sequences. The feasibility of individual steps of the method is described using the factor IX (F9) gene as a model system, and the mapping of several expressed sequences corresponding to a 330-kb **YAC** contg. DNA from human chromosome 6p21 is presented. In addn., the sequence, including an intron-exon junction, flanking a particular truncation event was obtained.

L7 ANSWER 3 OF 52 COPYRIGHT 1993 ACS
AN CA118(25):249077m
TI Genetic and physical mapping of the biglycan gene on the mouse X chromosome
SO Mamm. Genome, 4(1), 33-6
AU Chatterjee, Aurobindo; Faust, Cynthia J.; Herman, Gail E.
PY 1993
AN CA118(25):249077m
AB A human cDNA for biglycan (BGN) has recently been mapped to proximal Xq28. The murine locus, Bgn, was mapped .apprx.50 kb distal to DXPas8, using a combination of genetic mapping in an interspecific backcross of B6CBA-Aw-J/A-Bpa .times. Mus spretus and phys. mapping using pulsed field gel electrophoresis and anal. of murine **yeast artificial chromosomes** (**YACs**) contg. both DXPas8 and Bgn. The mapping studies also appear to exclude Bgn as a candidate gene for the bare patches (Bpa) mutation and for the **homologous** human disorder X-linked dominant chondrodysplasia punctata (CDPX2).

L7 ANSWER 4 OF 52 COPYRIGHT 1993 ACS
AN CA118(25):249043x
TI Clustered organization of **homologous** KRAB zinc-finger genes with enhanced expression in human T lymphoid cells
SO EMBO J., 12(4), 1363-74
AU Bellefroid, Eric J.; Marine, Jean Christophe; Reid, Thomas; Lecocq, Pierre J.; Riviere, Michele; Amemiya, Chris; Poncelet, Dominique A.; Coulie, Pierre G.; de Jong, Pieter; et al.
PY 1993

AN CA118(25):249043x
AB KRAB zinc-finger proteins (KRAB-ZFPs) constitute a large subfamily of ZFPs of the Krueppel C2H2 type. KRAB (Kruppel-assocd. box) is an evolutionary conserved protein domain found N-terminally with respect to the finger repeats. This report describes the characterization of a particular subgroup of highly related human KRAB-ZFPs. ZNF91 is one representative of this subgroup and contains 35 contiguous finger repeats at its C-terminus. Three mRNA isoforms with sequence identity to ZNF91 were isolated by the polymerase chain reaction. These encode proteins with a KRAB domain present, partially deleted or absent. Five genomic fragments were characterized, each encoding part of a gene: the ZNF91 gene or one or four distinct, related KRAB-ZFP genes. All exhibit a common exon/intron organization with the variant zinc finger repeats organized in a single exon and KRAB domain encoded by two sep. exons. This positioning of introns supports the hypothesis that the mRNA isoforms encoding polypeptide with variability in the KRAB domain could arise by alternative splicing. In situ chromosomal mapping studies and by anal. of fragments from a human genomic **yeast artificial chromosome** library contg. KRAB-ZFP genes showed that these genes occur in clusters. In particular, a gene complex contg. over 40 genes has been identified in chromosomal region 19p12-p13.1. These ZNF91-related gene probably arose late during evolution since no **homologous** genes are detected in the mouse and rat genomes. Although the transcription of members of this KRAB-ZFP gene subgroup is detectable in all human tissues, their expression is significantly higher in human T lymphoid cells.

L7 ANSWER 5 OF 52 COPYRIGHT 1993 ACS
AN CA118(17):162446g
TI Screening of DNA libraries in eukaryotic hosts using **homologous** recombination
SO PCT Int. Appl., 126 pp.
AU Treco, Douglas A.; Miller, Allan M.
PI WO 9303183 A1 18 Feb 1993
AI WO 91-US8679 21 Nov 1991
PY 1993
AN CA118(17):162446g
AB A method of screening a DNA library in an eukaryotic host that makes use of **homologous recombination** between a probe carrying a selectable marker and a target sequence is described. The host carrying the library is transformed with a non-replicating DNA fragment carrying the target sequence flanking the selectable marker. After allowing **homologous recombination** to take place the bank is selected for cells retaining the selectable marker. The preferred host for this method is Saccharomyces cerevisiae or Schizosaccharomyces pombe. A no. of **YAC** vectors for this use are described. The use of the method to identify a no. of human genes in a **YAC** bank is described.

L7 ANSWER 6 OF 52 COPYRIGHT 1993 ACS
AN CA118(15):140830a
TI Extension of yeast artificial chromosomes by cosmid multimers
SO Nucleic Acids Res., 21(3), 767-8
AU Davies, Nicholas P.; Bruggemann, Marianne

PY 1993
AN CA118(15):140830a
AB A rapid technique for the targeted extension of **YAC** clones using human DNA contained on cosmids is described. The method takes advantage of the integration mechanism of exogenous DNA into yeast, which allow the targeting of the cosmid DNA to be mediated by **homologous** sequences (i) within the insert, such as a cosmid clone which overlaps the DNA cloned in the **YAC**, and (ii) within the vector sequence present on the **YAC** arms and the cosmid vector itself. The extension of a 300 kb **YAC** up to 800 kb by adding excess DNA of a 50 kb cosmid clone is demonstrated.

L7 ANSWER 7 OF 52 COPYRIGHT 1993 ACS
AN CA118(13):118287g
TI Targeted deletion of chromosomal DNA using repetitive DNA-containing constructs
SO PCT Int. Appl., 24 pp.
AU Kucherlapati, Raju
PI WO 9301292 A1 21 Jan 1993
AI WO 92-US5409 26 Jun 1992
PY 1993
AN CA118(13):118287g
AB A method of introducing a mutation into a chromosome in host cells comprises transforming the cells with a construct contg. DNA **homologous** to a target site and joined to repetitive DNA and allowing **homologous recombination** to occur. Alternatively, yeast contg. a **YAC** with a chromosomal fragment may be transformed with the above construct, and, after **recombination** has occurred, the chromosomal fragment may be introduced into the target cells to cause mutagenesis. A construct contg. a human Factor IX gene fragment and a LINE1 element was used to mutagenize the Factor IX gene on a **YAC** in Saccharomyces cerevisiae. A series of nested deletion mutations was obtained.

L7 ANSWER 8 OF 52 COPYRIGHT 1993 ACS
AN CA118(9):74560j
TI The telomeric 60 kb of chromosome arm 4p is **homologous** to telomeric regions on 13p, 15p, 21p, and 22p
SO Genomics, 14(2), 350-6
AU Youngman, Sandra; Bates, Gillian P.; Williams, Sarah; McClatchey, Andrea I.; Baxendale, Sarah; Sedlacek, Zdenek; Altherr, Michael; Wasmuth, John J.; MacDonald, Marcy E.; et al.
PY 1992
AN CA118(9):74560j
AB A telomere **YAC** clone contg. the most distal 115 kb of chromosome arm 4p has been previously isolated. This clone is of particular interest as it spans a potential candidate region for the Huntington disease gene. The **YAC** was subcloned into a phage vector, and a high-resoln. restriction map extending to within 13 kb of the telomere was constructed. In situ hybridization of the **YAC** to human metaphase spreads gives a peak of hybridization on 4pter but also an increase in the no. of signals close to several other telomeres. Where possible, these results were investigated further by the hybridization of probes from the **YAC** to somatic cell hybrids contg. single human chromosomes. This anal. indicates that the most telomeric 60 kb of chromosome arm 4p is **homologous** to telomeric regions on 13p, 15p, 21p, and 22p.

The extent of this homol. makes it less likely that the mutation for Huntington's disease is located within the telomere **YAC** clone.

L7 ANSWER 9 OF 52 COPYRIGHT 1993 ACS
AN CA118(9):74470e
TI Creation of a yeast artificial chromosome fragmentation vector based on lysine-2
SO Genet. Anal.: Tech. Appl., 9(3), 86-90
AU Lewis, Brian C.; Shah, Neil P.; Braun, Benjamin S.; Denny, Christopher T.
PY 1992
AN CA118(9):74470e
AB **Yeast artificial chromosome** (**YAC**) fragmentation vectors have proven to be useful reagents for mapping and modifying **YAC** clones through **homologous recombination**. Such vectors can be employed to remove a noncontiguous sequence present in chimeric **YAC** clones. However, previous fragmentation vectors have been based on auxotrophies not present in the background of most **recombinant YAC** libraries, which therefore makes them difficult to use. An acentric **YAC** deletion vector pBCL was constructed that is fully compatible with AB 1380, the yeast host used in the majority of human total genomic **YAC** libraries. Transformation of three unrelated **YACs** with linearized pBCL yielded deriv. clones with the expected phenotype at rates of 27%-49%. Anal. of a random cohort of these clones by pulsed-field gel electrophoresis and Southern blotting revealed single deleted structures in 31 of 34 clones. Using this vector, a nested set of deletion derivs. can efficiently and reliably be generated from human **recombinant YAC** clones.

L7 ANSWER 10 OF 52 COPYRIGHT 1993 ACS
AN CA118(7):53395u
TI Mapping of the formin gene and exclusion as a candidate gene for the autosomal recessive form of limb-girdle muscular dystrophy
SO Hum. Mol. Genet., 1(8), 621-4
AU Richard, Isabelle; Broux, Odile; Hillaire, Dominique; Cherif, Dorra; Fougerousse, Francoise; Cohen, Daniel; Beckmann, Jacques S.
PY 1992
AN CA118(7):53395u
AB Limb-Girdle MUscular Dystrophy (LGMD) is a myopathy with clin. and transmission heterogeneity. The recessive form, LGMD2, has been recently mapped by linkage anal. to 15q. As an attempt to identify the gene involved in this pathol., the authors tested as candidate gene the LD locus, called LD for limb deformity. This gene has recently been identified and mapped to chromosome 15q13-q14. It is **homologous** to the murine formin gene which is localized to mouse chromosome 2. Mutations in this murine gene have been shown to cause limb deformity and kidney defect. **YAC** clones contg. the LD gene were isolated and utilized to confirm the cytogenetic localization. Internal DNA polymorphisms of the LD locus were analyzed in LGMD2 and CEPH families. The LD gene was mapped between the alpha cardiac actin gene and the D15S24 locus. Crossovers between the LGMD2 and the LD loci excluded the LD gene as a candidate for LGMD2.

L7 ANSWER 11 OF 52 COPYRIGHT 1993 ACS
AN CA118(7):53301k
TI Characterization by yeast artificial chromosome cloning of the linked apolipoprotein(a) and plasminogen genes and identification of the apolipoprotein(a) 5' flanking region
SO Proc. Natl. Acad. Sci. U. S. A., 89(23), 11584-8
AU Malgaretti, N.; Acquati, F.; Magnaughi, P.; Bruno, L.; Pontoglio, M.; Rocchi, M.; Saccone, S.; Della Valle, G.; D'Urso, M.; et al.
PY 1992
AN CA118(7):53301k
AB The apoprotein(a) [apo(a)] gene encodes a protein component of the circulating lipoprotein(a) [Lp(a)]. The apo(a) gene is highly **homologous** to the plasminogen gene. It encodes one of the most polymorphic human proteins, due to variability in the no. of repetitions of structures called kringles. In addn., Lp(a) levels vary among individuals by more than two orders of magnitude, the high levels being highly correlated with predisposition to early atherosclerotic disease. To better understand the genetics and function of the apo(a) gene, DNA fragments comprising the linked apo(a) and plasminogen genes and other members of the plasminogen family were cloned in **yeast artificial chromosome** vectors. By a combination of pulsed-field gel electrophoresis and genome walking expts., the 5' portion and flanking regions of the apo(a) gene were identified.

L7 ANSWER 12 OF 52 COPYRIGHT 1993 ACS
AN CA118(5):33951u
TI Gene manipulation and expression using genomic elements
SO PCT Int. Appl., 56 pp.
AU Sherwin, Stephen; Klapholz, Sue; Skoultchi, Arthur
PI WO 9219255 A1 12 Nov 1992
AI WO 92-US3686 5 May 1992
PY 1992
AN CA118(5):33951u
AB A method for expression of a mammalian target gene comprises integrating a target gene-amplifiable gene construct into the expression host genome, amplification of the target gene, and growth of the transformed host cells for prodn. of the desired protein. The target gene-amplifiable gene construct is prepd. by **homologous recombination** in yeast cells using a **YAC** contg. the target gene and an amplifiable gene (such as the dihydrofolate reductase gene) flanked by target gene sequence(s). **YAC** targeting vectors for activating the expression of the FSH-.beta. gene were prepd. and the gene expression in the CHO cells was shown.

L7 ANSWER 13 OF 52 COPYRIGHT 1993 ACS
AN CA118(1):1784a
TI Assignment of mouse .alpha.-2-macroglobulin gene to chromosome 6 band F1-G3
SO Mamm. Genome, 3(8), 469-71
AU Hilliker, Carl; Overbergh, Lut; Petit, Paul; Van Leuven, Fred; Van den Berghe, Herman
PY 1992
AN CA118(1):1784a
AB .alpha.-2-**Macroglobulin** (A2M) is a major proteinase inhibitor and scavenger in the circulation of humans.

Homologous proteins are present in all other vertebrates examd. Structurally, proteinase inhibitors of the .alpha. macroglobulin family (AM) are characterized by a subunit structure contg. about 1500 amino acids, with three specific structural features: a bait region, an internal thioester, and a receptor-binding domain. The authors included the bait region because it represents the most specific sequence that distinguishes it from other members of the murinoglobulin family. The 1.7-kb probe used for the isotopic in situ hybridization was generated by means of the polymerase chain reaction (PCR) essentially as described previously (C. Hilliker et al., 1991). Anal. of 85 metaphases from the NMRI strain yielded 173 grains (approx. 2.0 grains/metaphase). Significant hybridization was found on chromosome (Chr) 6 (34/173 grains or 1.6%), with grains randomly distributed over the other chromosomes. For the Robertsonian translocation Rb(6;15)1Ald, 75 metaphases were scored for 162 grains (approx. 2.2 grains/metaphase). Again the hybridization signal was predominantly localized on Chr 6 (35/162 grains or 21%), with the rest of the grains being randomly distributed over all the chromosomes. The assignment to Chr 6 is evidenced by 64.7% (22/34; Fig. 1a) of the grains localizing to the region 6 F1-G3 in the NMRI strain and 72% (25/35) of the grains in the same region for the Robertsonian translocation Rb(6.15)1Ald. In addn., fluorescent in situ hybridization (FISH) with the YAC clone, contg. the A2M gene, showed hybridization signal in the telomeric region of mouse Chr 6 in the reported area of localization, with about 85% of the metaphases displaying the yellow fluorescent signal on both chromatids of the two Chr 6 homologs. These results therefore support the localization of the mouse A2M gene to Chr 6 F1-G3.

L7 ANSWER 14 OF 52 COPYRIGHT 1993 ACS
AN CA117(25):246234d
TI The selective isolation of novel cDNAs encoded by the regions surrounding the human interleukin 4 and 5 genes
SO Nucleic Acids Res., 20(19), 5173-9
AU Morgan, John G.; Dolganov, Gregory M.; Robbins, Sabrina E.; Hinton, Linda M.; Lovett, Michael
PY 1992
AN CA117(25):246234d
AB Modifications to direct cDNA selection were developed that allow the rapid and reproducible isolation of low abundance cDNAs encoded by large genomic clones. Biotinylated, cloned genomic DNAs are hybridized in soln. with amplifiable cDNAs. The genomic clones and attached cDNAs are captured on streptavidin-coated magnetic beads, the cDNAs are eluted and amplified. This protocol was applied to a 425-kb **YAC** that contains the human IL4 and IL5 genes. After 2 cycles of enrichment 24 cDNAs were evaluated, all of which were **homologous** to the **YAC**. DNA sequencing revealed that 9 cDNAs were 100% **homologous** to the interferon regulatory factor 1 (IRF1) gene. Six clones were 70% **homologous** to the murine P600 gene, which is coexpressed with IL4 and IL5 in mouse Th2 cells. The 9 remaining clones were unique within the sequence databases and were non redundant. All of the selected cDNAs were initially present at very low abundance and were enriched by as much as 100,000-fold in 2 cycles of enrichment. This modified selection technique should be readily applicable to the isolation of many candidate disease loci as well as the

derivation of detailed transcription maps across large genomic regions.

L7 ANSWER 15 OF 52 COPYRIGHT 1993 ACS
AN CA117(23):227554s
TI The human Y chromosome: overlapping DNA clones spanning the euchromatic region
SO Science (Washington, D. C., 1883-), 258(5079), 60-6
AU Foote, Simon; Vollrath, Douglas; Hilton, Adrienne; Page, David C.
PY 1992
AN CA117(23):227554s
AB The human Y chromosome was phys. mapped by assembling 196 **recombinant** DNA clones, each contg. a segment of the chromosome, into a single overlapping array. This array included more than 98 percent of the euchromatic portion of the Y chromosome. First, a library of **yeast artificial chromosome** (**YAC**) clones was prepd. from the genomic DNA of a human XYYYY male. The library was screened to identify clones contg. 160 sequence-tagged sites and the map was then constructed from this information. In all, 207 Y-chromosomal DNA loci were assigned to 127 ordered intervals on the basis of their presence or absence in the **YAC**'s, yielding ordered landmarks at an av. spacing of 220 kilobases across the euchromatic region. The map reveals that Y-chromosomal genes are scattered among a patchwork of X-**homologous**, Y-specific repetitive, and single-copy DNA sequences. This map of overlapping clones and ordered, densely spaced markers should accelerate studies of the chromosome.

L7 ANSWER 16 OF 52 COPYRIGHT 1993 ACS
AN CA117(23):227319u
TI A yeast artifical chromosome contig encompassing the type 1 neurofibromatosis gene
SO Genomics, 13(3), 672-80
AU Marchuk, Douglas A.; Tavakkol, Roxanne; Wallace, Margaret R.; Brownstein, Bernard H.; Taillon-Miller, Patricia; Fong, Chin To; Legius, Eric; Andersen, Lone B.; Glover, Thomas W.; Collins, Francis S.
PY 1992
AN CA117(23):227319u
AB The **yeast artificial chromosome** (**YAC**) system allows the direct cloning of large regions of the genome. A **YAC** contig map of .apprx.700 kb encompassing the region surrounding the type 1 neurofibromatosis (NF1) locus on 17q11.2 has been constructed. A single **YAC** contg. the entire NF1 locus has been constructed by **homologous recombination** in yeast. In the process of contig construction a novel method of **YAC** end rescue has been developed by **YAC** circularization in yeast and plasmid rescue in bacteria. **YACs** contg. homol. to the NF1 region but mapping to another chromosome have also been discovered. Sequences of portions of the **homologous** locus indicate that this other locus is a nonprocessed pseudogene.

L7 ANSWER 17 OF 52 COPYRIGHT 1993 ACS
AN CA117(21):206162x
TI Molecular linkage of the human CTLA4 and CD28 Ig-superfamily genes

in yeast artificial chromosomes
SO Genomics, 13(3), 856-61
AU Buonavista, Nathalie; Balzano, Christine; Pontarotti, Pierre; Le Paslier, Denis; Goldstein, Pierre
PY 1992
AN CA117(21):206162x
AB CD28 and CTLA4 are structurally **homologous** single-V-domain mols. of the Ig superfamily, the genes of which comap on the same chromosomal bands in mouse and man. Using polymerase chain reactions, the authors isolated six **yeast artificial chromosome** (**YAC**) clones pos. for CTLA4 and/or CD28 from a human-DNA-contg. **YAC** library. Two double-pos. clones, 365 and 550 kb long, resp., were further studied. Detailed restriction enzyme maps showed that one of these **YACs** was nested in the other, that they both bore the same CD28- and CTLA4-hybridizing fragments, that similar fragments were seen in genomic DNA, and that the distance between the CD28 and CTLA4 genes was at most 150 kb and at least 25 kb. A CpG island was found between these genes. These results provide a high-resoln. est. of the phys. distance between the CD28 and CTLA4 genes and constitute a basis for the isolation of neighboring structures.

L7 ANSWER 18 OF 52 COPYRIGHT 1993 ACS
AN CA117(17):165073y
TI A 1.6-Mb contig of **yeast artificial chromosomes** around the human factor VIII gene reveals three regions **homologous** to probes for the DXS115 locus and two for the DXYS64 locus
SO Am. J. Hum. Genet., 51(1), 66-80
AU Freije, Diha; Schlessinger, David
PY 1992
AN CA117(17):165073y
AB Two **yeast artificial chromosome** (**YAC**) libraries were screened for probes in Xq28, around the gene for coagulation factor VIII (F8). A set of 30 **YACs** were recovered and assembled into a contig spanning at least 1.6 Mb from the DXYS64 locus to the glucose 6-phosphate dehydrogenase gene (G6PD). Overlaps among the **YACs** were detd. by several fingerprinting techniques and by addnl. probes generated from **YAC** inserts by using Alu-vector or ligation-mediated PCR. Anal. of more than 30 probes and sequence-tagged sites (STSs) made from the region revealed the presence of several **homologous** genomic segments. For example, a probe for the DXYS64 locus, which maps less than 500 kb 5' of F8, detects a similar but not identical locus between F8 and G6PD. Also, a probe for the DXS115 locus detects at least 3 identical copies in this region, 1 in intron 22 of F8 and at least 2 more, which are upstream of the 5' end of the gene. Comparisons of genomic and **YAC** DNA suggest that the multiple loci are not created artifactually during cloning but reflect the structure of uncloned human DNA. On the basis of these data, the most likely order for the loci analyzed is tel-DXYS61-DXYS64-(DXS115-3-DXS115-2)-5'F8-(DXS115-1)-3'F8-G6PD.

L7 ANSWER 19 OF 52 COPYRIGHT 1993 ACS
AN CA117(15):144536m
TI **Recombinational** repair of diverged DNAs: a study of homoeologous chromosomes and mammalian **YACs** in yeast

SO Mol. Gen. Genet., 234(1), 65-73
AU Resnick, Michael A.; Zgaga, Zoran; Hieter, Philip; Westmoreland, James; Fogel, Seymour; Nilsson-Tillgren, Torsten
PY 1992
AN CA117(15):144536m
AB **Recombinational** repair is the means by which DNA double-strand breaks (DSBs) are repaired in yeast. DNA divergence between chromosomes was shown previously to inhibit repair in diploid G1 cells, resulting in chromosome loss at low nonlethal doses of ionizing radiation. Furthermore, 15-20% divergence prevents meiotic **recombination** between individual pairs of Saccharomyces cerevisiae and S. carlsbergensis chromosomes in an otherwise S. cerevisiae background. Based on anal. of the efficiency of DSB-induced chromosome loss and direct genetic detection of intragenic **recombination**, the authors concluded that limited DSB **recombinational** repair can occur between homoeologous chromosomes. There is no difference in loss between a repair-proficient Pms+ strain and a mismatch repair mutant, pms1. Since DSB **recombinational** repair is tolerant of diverged DNAs, this type of repair could lead to novel genes and altered chromosomes. The sensitivity to DSB-induced loss of 11 individual **yeast artificial chromosomes** (**YACs**) contg. mouse or human (chromosome 21 or HeLa) DNA was detd. **Recombinational** repair between a pair of **homologous** HeLa **YACs** appears as efficient as that between **homologous** yeast chromosomes in that there is no loss at low radiation doses. Single **YACs** exhibited considerable variation in response, although the response for individual **YACs** was highly reproducible. Based on the results with the yeast homeologous chromosomes, the authors proposed that the potential exists for intra-**YAC** **recombinational** repair between diverged repeat DNA and that the extent of repair is dependent upon the amt. of repeat DNA and the degree of divergence. The sensitivity of **YACs** contg. mammalian DNA to ionizing radiation-induced loss may thus be an indicator of the extent of repeat DNA.

L7 ANSWER 20 OF 52 COPYRIGHT 1993 ACS
AN CA117(11):105483x
TI An optimized Alu-PCR primer pair for human-specific amplification of YACs and somatic cell hybrids
SO Hum. Mol. Genet., 1(2), 121-2
AU Tagle, Danilo A.; Collins, Francis S.
PY 1992
AN CA117(11):105483x
AB Inter-Alu PCR has been invaluable in the characterization and anal. of somatic cell hybrids and **YACs**. A pair of improved Alu PCR primers designed from the aligned consensus sequences of known subfamilies is reported. The two primers were designed such that: 1) they prime close to the 5' and 3' ends of Alu elements, thus minimizing the repetitive Alu content of the inter-Alu PCR products, 2) they recognize and prime from the highly conserved regions of Alu elements, thus amplifying the more abundantly dispersed but anciently inserted and more diverged Alu elements, 3) there is minimal mispriming to **homologous** sequences internal to the dimeric Alu element, and 4) both can be used together in the PCR reaction so amplification takes place irresp. of the orientation of

adjacent Alu elements. The Alu-5' primer has the sequence 5'-GGATTACAGGCGTGAGCCAC-3' and the Alu-3' primer has the sequence 5'-GATCGCGCCACTGCACTCC-3'.

L7 ANSWER 21 OF 52 COPYRIGHT 1993 ACS
AN CA117(11):105377r
TI Targeted integration of neomycin into yeast artificial chromosomes (YACs) for transfection into mammalian cells
SO Nucleic Acids Res., 20(12), 2971-6
AU Riley, J. H.; Morten, J. E. N.; Anand, R.
PY 1992
AN CA117(11):105377r
AB Vectors have been constructed for the introduction of the neomycin resistance gene (neo) into the left arm, right arm or human insert DNA of **yeast artificial chromosomes** (**YACs**) by **homologous recombination**. These vectors contain a yeast selectable marker lys-2, i.e. the .alpha.-aminoadipidate reductase gene, and a mammalian selection marker, neo, which confers G418 resistance. The vectors can be used to modify **YACs** in the most commonly used yeast strain for **YAC** library construction, AB1380. Specific targeting can be carried out by transfection of restriction endonuclease treated linear plasmids, with highly specific **recombinogenic** ends, into the **YAC** contg. yeast cells. Anal. of targeted **YACs** confirmed that all three vectors can target correctly in yeast. Introduction of one of the targeted **YACs** into V79 (Chinese hamster fibroblast) cells showed complete and intact transfer of the **YAC**.

L7 ANSWER 22 OF 52 COPYRIGHT 1993 ACS
AN CA117(9):84386a
TI Targeted alterations in yeast artificial chromosomes for inter-species gene transfer
SO Nucleic Acids Res., 20(11), 2693-8
AU Davies, Nicholas P.; Rosewell, Ian R.; Bruggemann, Marianne
PY 1992
AN CA117(9):84386a
AB In order to facilitate alterations of large DNA mols. for their introduction into mammalian cells the authors characterized the mechanism of site-specific modifications in **yeast artificial chromosomes** (**YACs**). Newly developed yeast integration vectors with dominant selectable marker genes allow targeted integration into left (centromeric) and right (noncentromeric) **YAC** arms as well as alterations to the human derived insert DNA. In transformation expts., integration proceeds exclusively by **homologous recombination** although yeast prefers linear ends of homol. for predefined insertions. Targeted regions can be rescued which expedite the cloning of internal human sequences and the identification of 5' and 3' **YAC**/insert borders. Integration of the neomycin resistance gene into various parts of the **YAC** allowed the transfer and stable integration of large DNA mols. into a variety of mammalian cells including embryonic stem cells.

L7 ANSWER 23 OF 52 COPYRIGHT 1993 ACS
AN CA117(5):42865a
TI Meiotic recombination and segregation of human-derived artificial

chromosomes in Saccharomyces cerevisiae
SO Proc. Natl. Acad. Sci. U. S. A., 89(12), 5296-300
AU Sears, Dorothy D.; Hegemann, Johannes H.; Hieter, Philip
PY 1992
AN CA117(5):42865a
AB The authors developed a system that utilizes human DNA-derived **yeast artificial chromosomes** (**YACs**) as marker chromosomes to study factors that contribute to the fidelity of meiotic chromosome transmission. Since aneuploidy for the **YACs** does not affect spore viability, different classes of meiotic missegregation can be scored accurately in four-viable-spore tetrads including precocious sister sepn., meiosis I nondisjunction, meiotic chromatid loss, and meiosis II nondisjunction. Segregation of the **homologous** pair of 360-kilobase marker **YACs** was shown to occur with high fidelity in the first meiotic division and was assocd. with a high frequency of **recombination** within the human DNA segment. By using this exptl. system, a series of **YAC** deletion derivs. ranging in size from 50 to 225 kilobases was analyzed to directly assess the relationship between meiotic **recombination** and meiosis I disjunction in a genotypically wild-type background. The relationship between phys. distance and **recombination** frequency within the human DNA segment was measured to be comparable to that of endogenous yeast chromosomal DNA, ranging from <2.0 to 7.7 kilobases/centimorgan. Phys. anal. of **recombinant** chromosomes detected no unequal crossing-over at dispersed repetitive elements distributed along the **YACs**. **Recombination** between **YACs** contg. unrelated DNA segments was not obsd. Furthermore, the segregational data indicated that meioses in which **YAC** pairs failed to **recombine** exhibited dramatically increased levels of meiosis I missegregation, including both precocious sister chromatid sepn. and nondisjunction.

L7 ANSWER 24 OF 52 COPYRIGHT 1993 ACS
AN CA117(3):21282t
TI Reconstruction of the 2.4 Mb human DMD-gene by **homologous YAC recombination**
SO Hum. Mol. Genet., 1(1), 19-28
AU Den Dunnen, J. T.; Grootscholten, P. M.; Dauwerse, J. G.; Walker, A. P.; Monaco, A. P.; Butler, R.; Anand, R.; Coffey, A. J.; Bentley, D. R.; et al.
PY 1992
AN CA117(3):21282t
AB The human dystrophin gene, mutations of which cause Duchenne and Becker muscular dystrophy, measures 2.4 Mb. This size seriously limits its cloning as a single DNA fragment and subsequent in-vitro expression studies. Stepwise in-vivo **recombination** between overlapping **yeast artificial chromosomes** (**YACs**) was used to reconstruct the dystrophin gene. The **recombinant YACs** are mitotically stable upon propagation in haploid yeast cells. In contrast, specific combinations of **YACs** display a remarkable mitotic and meiotic instability in diploid cells. Non-disjunction is rare for overlapping **YACs**, but increases upon sporulation of diploid cells contg. non-overlapping mols. This feature was exploited in a three-point **recombination** to bridge a 280

kb gap between two non-overlapping **YACs** for which no **YAC** of proper polarity existed. The largest **recombinant YAC** measures 2.3 Mb and contains and entire muscle specific DMD-gene with the exception of a 100 kb region contg. the in-frame exon 60. The latter segment has a high tendency to undergo deletions in multi-mol. interactions, probably due to the presence of as yet unidentified instability-enhancing sequences. Fluorescent in situ hybridizations confirmed that the 2.3 Mb DMD **YAC** contained Xp21-sequences only and indicated a compact tertiary structure of the DMD-gene in interphase lymphocyte nuclei. It is concluded that the yeast system is a flexible, efficient and generally applicable tool to reconstruct or build genomic regions from overlapping **YAC** constituents. Its application to the human dystrophin gene has provided many possibilities for future studies.

L7 ANSWER 25 OF 52 COPYRIGHT 1993 ACS
AN CA116(21):208726n
TI Yeast artificial chromosomes (YACs) and the analysis of complex genomes
SO Trends Biotechnol., 10(1-2), 35-40
AU Anand, Rakesh
PY 1992
AN CA116(21):208726n
AB A review with 58 refs. The development of **yeast artificial chromosome** (**YAC**) cloning vectors capable of carrying several hundred kilobase-pairs of DNA insert has greatly facilitated the study of complex genomes, and the cloning of large genes as single fragments. In addn., the ability to manipulate **YAC** sequences by **homologous recombination** makes this system extremely useful for the generation of disease models.

L7 ANSWER 26 OF 52 COPYRIGHT 1993 ACS
AN CA116(19):188896n
TI Analysis of the human regulators of complement activation (RCA) gene cluster with yeast artificial chromsomes (YACs)
SO Genomics, 12(2), 289-300
AU Hourcade, Dennis; Garcia, Andrew D.; Post, Theodore W.; Taillon-Miller, Patricia; Holers, V. Michael; Wagner, Lynne M.; Bora, Nalini S.; Atkinson, John P.
PY 1992
AN CA116(19):188896n
AB The human regulators of complement activation gene cluster (RCA cluster) have been partially characterized with **yeast artificial chromosomes** (**YACs**). While the data confirm many points previously elucidated, the finer resoln. of **YAC** mapping has allowed the discovery and/or localization of partial gene duplications, the detn. of gene orientations, and the measurement of gaps between known genes. Here nine overlapping **YACs** that encompass a genomic region of 800 kb, encoding four RCA genes and three gene-like elements, are described. The encoded genes and two of the gene-like elements share the same orientation and are ordered (5' to 3') DAF, CR2, CR1, MCP-like, CR1-like, and MCP. A C4bp-like region lies upstream from DAF and is likely to correspond to one recently obsd. by F. Pardo-Manuel, (1990). MCP-like, a new genetic element, was discovered and found to

be **homologous** to the 5' portion of the MCP gene. Two large gaps of 85 kb (between CR2 and DAF) and 110 kb (between DAF and the C4bp-like element) could carry addnl. RCA genes. The arrangement of CR1, MCP-like, CR1-like, and MCP, in that order, strongly suggests that this region was generated by a single duplication of neighboring CR1/CR1-like and MCP/MCP-like forerunners. The RCA **YACs** will now serve as convenient DNA sources for the subcloning and further characterization of this region.

L7 ANSWER 27 OF 52 COPYRIGHT 1993 ACS
AN CA116(17):167144a
TI Transfer of yeast artificial chromosomes from yeast to mammalian cells
SO BioEssays, 13(10), 545-50
AU Huxley, Clare; Gnirke, Andreas
PY 1991
AN CA116(17):167144a
AB A review with 40 refs. Human DNA can be cloned as **yeast artificial chromosomes** (**YACs**), each of which contains several hundred kilobases of human DNA. This DNA can be manipulated in the yeast host using **homologous recombination** and yeast selectable markers. In relatively few steps it is possible to make virtually any change in the cloned human DNA from single base pair changes to deletions and insertions. In order to study the function of the cloned DNA and the effects of the changes made in the yeast, the human DNA must be transferred back into mammalian cells. Recent expts. indicate that large genes can be transferred from the yeast host to mammalian cells in tissue culture and that the genes are transferred intact and are expressed. Using the same methods it may soon be possible to transfer **YAC** DNA into the mouse germ line so that the expression and function of genes cloned in **YACs** can be studied in developing and adult mammalian animals.

L7 ANSWER 28 OF 52 COPYRIGHT 1993 ACS
AN CA116(13):126187y
TI Cloning of the essential myotonic dystrophy region and mapping of the putative defect
SO Nature (London), 355(6360), 548-51
AU Aslanidis, Charalampos; Jansen, Gert; Amemiya, Chris; Shutler, Gary; Mahadevan, Mani; Tsilfidis, Catherine; Chen, Chira; Alleman, Jennifer; Wormskamp, Nicole G. M.; et al.
PY 1992
AN CA116(13):126187y
AB Myotonic dystrophy is a common dominant disorder (global incidence of 1:8000) with variable onset and a protean nature of symptoms mainly involving progressive muscle wasting, myotonia and cataracts: to define the mol. defect, this study cloned the essential region of chromosome 19q13.3, including proximal and distal markers in a 700-kilobase contig formed by overlapping cosmids and **yeast artificial chromosomes** (**YACs**). The central part of the contig bridges an area of about 350 kilobases between two new flanking crossover borders. This segment has been extensively characterized through the isolation of five **YAC** clones and the subsequent subcloning in cosmids from which a detailed EcoRI, HindIII, MluI and NotI restriction map has been derived. Two genomic probes and two **homologous** cDNA probes

were isolated using the cosmids. These probes are all situated within .apprx.10 kilobases of genomic DNA and detect an unstable genomic segment in myotonic dystrophy patients. The length variation in this segment shows similarities to the instability seen at the fragile X locus. The phys. map location and the genetic characteristics of the length polymorphism is compatible with a direct role in the pathogenesis of myotonic dystrophy.

L7 ANSWER 29 OF 52 COPYRIGHT 1993 ACS
AN CA116(13):122639z
TI **Homologous** recombination method for identifying and isolating DNA fragments from DNA libraries in eukaryotic cells
SO PCT Int. Appl., 77 pp.
AU Treco, Douglas A.; Miller, Allan M.
PI WO 9201069 A1 23 Jan 1992
AI WO 91-US4926 12 Jul 1991
PY 1992
AN CA116(13):122639z
AB A method for selecting DNA fragments in an eukaryotic host by forced integration of a selectable marker into the sequence of interest is described. A DNA fragment carrying a selectable marker and a sequence long enough to direct **homologous recombination** to take place is introduced into the host carrying the bank. The bank is selected for stable transformants carrying the marker. This method is useful for chromosome walking and genetic mapping. It allows screening for many specific sequences simultaneously and storage of libraries as a pool of clones rather than as individuals; and it speeds up the library screening process. The method was illustrated using yeast ARG4- contg. DNA from human white blood cells on **YACs** carrying the selectable markers TRP1 and URA3. Targeting plasmid p184DLARG contg. the yeast ARG4 gene and a bacterial origin of replication was prepd. and a DNA fragment contg. the 5' flanking region of the .epsilon.-globin gene was inserted into it. Clones contg. the desired globin sequences were identified by growth on medium lacking uracil, tryptophan, and arginine.

L7 ANSWER 30 OF 52 COPYRIGHT 1993 ACS
AN CA116(11):104294d
TI A natural killer cell granule protein that induces DNA fragmentation and apoptosis
SO J. Exp. Med., 175(2), 553-66
AU Shi, Lianfa; Kraut, Ricky P.; Aebersold, Ruedi; Greenberg, Arnold H.
PY 1992
AN CA116(11):104294d
AB The purifn. is reported from a rat natural killer (RNK) large granular lymphocyte leukemia of a 32-kD granule protein that induces rapid DNA fragmentation and apoptosis. The protein, named fragmentin, was capable of causing DNA from intact **YAC**-1 cells to be cleaved into oligonucleosomal-sized fragments and producing severe chromatin condensation within 1 h. Amino acid sequence of tryptic peptides indicated that fragmentation was highly **homologous** to the NK and T cell granule serine proteases RNK protease 1 and mouse cytotoxic T cell protease I (CCPI)/granzyme B. Preincubation with the serine esterase inhibitor 3,4-dichloroisocoumarin blocked fragmentin-induced DNA damage, but had no effect on cytolysin. Fragmentin activity against 4 lymphoma

target cells was completely dependent on the presence of cytolysin. Fragmentin produced rapid membrane damage as well as DNA fragmentation at nonlytic cytolysin doses, suggesting that fragmentin activity was not limited to its effects on the nucleus. Fragmentin and cytolysin activity were completely inhibited by EGTA, indicating the process was Ca2+ dependent. A role for cytolysin in endocytosis of fragmentin was suggested by the observation that treatment of YAC-1 with cytochalasin B or sodium azide and 2-deoxyglucose blocked DNA fragmentation but not cytolysin activity. A 30-kD N.alpha.-CBZ-L-lysine thiobenzyl esterase, which copurified with fragmentin, was inactive on its own but could synergistically amplify the DNA damage induced by fragmentin in the presence of cytolysin. Fragmentin activity was not dependent on protein synthesis, as cycloheximide treatment of YAC-1 cells did not prevent DNA damage. It is postulated that fragmentin is the mol. mediator of NK cell-mediated DNA fragmentation and apoptosis.

L7 ANSWER 31 OF 52 COPYRIGHT 1993 ACS
AN CA116(3):19634v
TI Recognition of .beta.2-microglobulin-negative (.beta.2m-) T-cell blasts by natural killer cells from normal but not from .beta.2m- mice: nonresponsiveness controlled by .beta.2m- bone marrow in chimeric mice
SO Proc. Natl. Acad. Sci. U. S. A., 88(22), 10332-6
AU Hoeglund, Petter; Oehlen, Claes; Carbone, Ennio; Franksson, Lars; Ljunggren, Hans Gustaf; Latour, Ann; Koller, Beverly; Kaerre, Klas
PY 1991
AN CA116(3):19634v
AB The role of major histocompatibility complex (MHC) class I expression in control of the sensitivity of normal cells to natural killer (NK) cells was studied by the use of mutant mice made deficient for expression of .beta.2-microglobulin (.beta.2m) through homologous recombination in embryonal stem cells. T-cell blasts from .beta.2m-deficient (.beta.2m -/-) mice were killed by NK cells from normal mice in vitro, while .beta.2m +/- blasts were resistant. The .beta.2m defect also affected the NK effector cell repertoire: NK cells from .beta.2m -/- mice failed to kill .beta.2m -/- blasts, while they retained the ability to kill the prototype NK cell target lymphoma YAC-1, although at reduced levels. The inability to recognize .beta.2m -/- blasts could be transferred with .beta.2m -/- bone marrow to irradiated .beta.2m-expressing mice. In contrast, the development of CD8+ T cells (deficient in .beta.2m -/- mice) was restored in such chimera. Thus, loss of MHC class I/.beta.2m expression is sufficient to render normal cells sensitive to NK cells, and the same defect in the hemopoietic system of a mouse renders its NK cells tolerant to .beta.2m-deficient but otherwise normal cells. In the .beta.2m -/- mice, NK cells may be selected or educated by other bone marrow cells to tolerate the MHC class I deficiency. Alternatively, the specificity may be controlled directly by the class I mols. on the NK cells themselves.

L7 ANSWER 32 OF 52 COPYRIGHT 1993 ACS
AN CA116(1):1473g
TI High-efficiency yeast artificial chromosome fragmentation vectors
SO Gene, 106(1), 125-7
AU Pavan, William J.; Hieter, Philip; Sears, Dorothy; Burkhoff, Amanda;

Reeves, Roger H.
PY 1991
AN CA116(1):1473g
AB Chromosome fragmentation vectors (CFVs) are used to create deletion derivs. of large fragments of human DNA cloned as **yeast artificial chromosomes** (**YACs**). CFVs target insertion of a telomere sequence into the **YAC** via **homologous recombination** with Alu repetitive elements. This event results in the loss of all **YAC** sequences distal to the site of integration. A new series of CFVs has been developed. These vectors target fragmentation to both Alu and LINE human repetitive DNA elements. Recovery of deletion derivs. is 10-20-fold more efficient with the new vectors than with those described previously.

L7 ANSWER 33 OF 52 COPYRIGHT 1993 ACS
AN CA115(21):225404c
TI Rescue of end fragments of **yeast artificial chromosomes** by **homologous recombination** in yeast
SO Nucleic Acids Res., 19(18), 4943-8
AU Hermanson, Gary G.; Hoekstra, Merl F.; McElligott, David L.; Evans, Glen A.
PY 1991
AN CA115(21):225404c
AB **Yeast artificial chromosomes** (**YACs**) provide a powerful tool for the isolation and mapping of large regions of mammalian chromosomes. The authors developed a rapid and efficient method for the isolation of DNA fragments representing the extreme ends of **YAC** clones by the insertion of a rescue plasmid into the **YAC** vector by **homologous recombination**. Two rescue vectors were constructed contg. a yeast LYS2 selectable gene, a bacterial origin of replication, an antibiotic resistance gene, a polylinker contg. multiple restriction sites, and a fragment **homologous** to one arm of the pYAC4 vector. The end-cloning procedure involves transformation of the rescue vector into yeast cells carrying a **YAC** clone, followed by prepn. of yeast DNA and transformation into bacterial cells. The resulting plasmids carry end-specific DNA fragments up to 20 kb in length, which are suitable for use as hybridization probes, as templates for direct DNA sequencing, and as probes for mapping by fluorescence in situ hybridization. These vectors are suitable for the rescue of end-clones from any **YAC** constructed using a pYAC-derived vector. The authors demonstrate the utility of these plasmids by rescuing **YAC**-end fragments from a human **YAC** library.

L7 ANSWER 34 OF 52 COPYRIGHT 1993 ACS
AN CA115(21):225037k
TI He-T family DNA sequences in the Y chromosome of Drosophila melanogaster share homology with the X-linked Stellate genes
SO Chromosoma, 100(2), 118-24
AU Danilevskaya, O. N.; Kurenova, E. V.; Pavlova, M. N.; Bebekhov, D. V.; Link, Andrew J.; Koga, Akihiko; Vellek, Ann; Hartl, Daniel L.
PY 1991
AN CA115(21):225037k

AB The genome of D. melanogaster contains a class of repetitive DNA sequences called the He-T family, which is unusual in being confined to telomeric and heterochromatic regions. The specific He-T fragment designated Dm665 was cloned in yeast by selection for an autonomously replicating sequence (ARS). Dm665 contains a restriction fragment length polymorphism (RFLP) that is specific to males and thus derives from the Y chromosome. Deletion mapping using X-Y translocations indicates that sequences **homologous** to Dm665 occur in at least one major cluster in each arm of the Y chromosome. Among 20 **yeast artificial chromosome** (**YAC**) clones contg. Drosophila sequences **homologous** with Dm665, 4 clones derive from defined regions of the long arm of the Y and 2 from the short arm. The sequence of Dm665 is 2443 bp long, consists of 59% A + T, and contains no significant open reading frames or direct or inverted repeats. However, Dm665 contains a region of 650 bp that shares homol. with portions of the X-linked locus Stellate.

L7 ANSWER 35 OF 52 COPYRIGHT 1993 ACS
AN CA115(19):200250m
TI Integrative selection of human chromosome-specific yeast artificial chromosomes
SO Proc. Natl. Acad. Sci. U. S. A., 88(17), 7788-91
AU Pavan, William J.; Reeves, Roger H.
PY 1991
AN CA115(19):200250m
AB Human-specific integrative selection vectors (ISVs) were designed to optimize integration of a yeast-selectable marker specifically into **yeast artificial chromosomes** (**YACs**) derived from human but not mouse DNA. ISVs were transformed into a **YAC** genomic library constructed from DNA of a human-mouse somatic cell hybrid contg. chromosome 21 (HSA21) as the only human chromosome. One percent of the yeast in the original library contained HSA21-derived **YACs**; between 45% and 54% of the yeast recovered after transformation with ISV vectors contained human **YACs**. Integrative selection provides a rapid means of obtaining a high enriched population of human chromosome-specific **YACs** by eliminating the labor-intensive steps of isolating and screening primary transformants. The procedure is biased toward the selection of **YACs** that contain a large no. of targets for **homologous recombination**; thus, libraries constructed by this procedure will be composed primarily of the largest **YACs** in the population.

L7 ANSWER 36 OF 52 COPYRIGHT 1993 ACS
AN CA115(17):176659d
TI Rapid screening of a YAC library by pulsed-field gel Southern blot analysis of pooled YAC clones
SO Genomics, 10(3), 661-5
AU Mendez, Michael J.; Klapholz, Sue; Brownstein, Bernard H.; Gemmill, Robert M.
PY 1991
AN CA115(17):176659d
AB A new method for screening of **YAC** libraries is described. Individual **YACs** were pooled into groups of 384 clones and prepd. as samples suitable for pulsed-field gel electrophoresis. A

5-hit human **YAC** library contg. .apprx.60,000 clones was condensed into 150 such pools and chromosomal DNAs in each sample were sepd. on 3 pulsed field gels contg. 50 samples each. Southern blots prepd. from these gels were hybridized with probes of interest to identify pools contg. **homologous YACs**. Further purifn. was performed using std. colony hybridization procedures. Twenty-one probes used thus far have identified 47 pos. pools, and corresponding **YACs** have been purified from 28 of these. Some significant advantages of this method include avoidance of DNA sequence anal. and primer generation prior to **YAC** screening and the ability to handle the entire library on 3 filters. The screening approach described here permits rapid isolation of **YACs** corresponding to unsequenced loci and will accelerate establishment of **YAC** contigs for large chromosomal segments.

L7 ANSWER 37 OF 52 COPYRIGHT 1993 ACS
AN CA115(17):176617p
TI Vectors for inserting selectable markers in vector arms and human DNA inserts of yeast artificial chromosomes (YACs)
SO Gene, 103(1), 53-9
AU Srivastava, Anand K.; Schlessinger, David
PY 1991
AN CA115(17):176617p
AB To facilitate studies of gene expression and **homologous recombination**, plasmids have been developed which permit the insertion of neomycin resistance-encoding gene (NmR) into either the human DNA insert or the vector arm of a **yeast artificial chromosome** (**YAC**). To integrate into the **YAC** arm, the plasmid pRV1 contains a LYS2 (encoding .alpha.-aminoadipate reductase) gene for selection in the yeast host, and a NmR gene for subsequent selection after transfection of mammalian cells. These two sequences are bracketed by fragments of the URA3 gene (encoding orotidine-5'-phosphate decarboxylase) that can disrupt the URA3 gene in the **YAC** arm by **homologous recombination** in yeast. To integrate a selectable marker into the insert, the plasmid pRV2 contains a NmR gene and an intact copy of the URA3 gene, bracketed by segments of an L1 (LINEs) repetitive element. In this case, the vector has been designed for use with **YACs** that have already been fitted in the vector arm with a different marker (i.e., TK) that has dirsupted the URA3 gene in the vector arm. Selection is for the restoration of URA3 gene activity attendant on **recombination** into an L1 element in the **YAC** insert. Use of the vectors is illustrated with a **YAC** clone contg. ribosomal DNA.

L7 ANSWER 38 OF 52 COPYRIGHT 1993 ACS
AN CA115(17):176586c
TI Characterization and expression of the human leukocyte-common antigen (CD45) gene contained in yeast artificial chromosomes
SO Genomics, 10(3), 756-64
AU Fernandez-Luna, Jose L.; Matthews, R. James; Brownstein, Bernard H.; Schreiber, Robert D.; Thomas, Matthew L.
PY 1991
AN CA115(17):176586c
AB The leukocyte-common antigen (CD45) is a transmembane protein

tyrosine phosphatase expressed uniquely by cells of hematopoietic origin. There are multiple isoforms of CD45 that are generated by the variable use of 3 exons (exons 4-6). The use of the variable exons results in changes near the amino-terminus of the mature glycoprotein. The gene is located on chromosome 1 for both human and mouse in a region that is **homologous** between these 2 species. This conserved linkage group contains a no. of genes of immunol. interest, such as the genes for complement regulatory proteins and the FCG2 receptor. **Yeast artificial chromosomes** provide a vector system in which large fragments of foreign DNA can be isolated and are suited to long-range phys. mapping. To this end, 3 **yeast artificial chromosomes** contg. the human CD45 gene were isolated and characterized. They overlap to span 475 kb, establishing the largest phys. map for DNA within the conserved linkage group. The CD45 gene is entirely encoded within 1 **yeast artificial chromosome** clone as detd. by mapping with cDNA probes. A mouse B cell line transfected with this **YAC** clone expressed the low-mol.-wt. isoform of the protein into the cell surface. The size of the human CD45 gene was detd. to be .apprx.120 .+-. 10 kb.

L7 ANSWER 39 OF 52 COPYRIGHT 1993 ACS
AN CA115(13):128555v
TI Yeast artificial chromosomes: promises kept and pending
SO Genome Anal., 1(Genet. Phys. Mapp.), 83-120
AU Hieter, Philip; Connelly, Carla; Shero, James; McCormick, Mary Kay; Antonarakis, Stylianos; Pavan, William; Reeves, Roger
PY 1990
AN CA115(13):128555v
AB A review with many refs. of the development and application of **yeast artificial chromosome** (**YAC**) cloning methods for both the phys. mapping of genomes and the functional anal. of specific genes or DNA elements. The main topics discussed include: 1) the yeast life cycle, DNA-mediated transformation methodol. and characteristics, and the identification and characterization of structural components required for chromosome maintenance in Saccharomyces cerevisiae. This section provides a general background to those investigators who are working with artificial chromosomes in yeast for the first time. 2) The development and application of **YAC** cloning strategies to the phys. anal. of complex genomes. These technologies are sufficiently developed and proven to be crucial to future large-scale genome-mapping projects. 3) Applications of **homologous-recombination**-based manipulation of specific **YAC** clones and strategies for the subsequent transfer of **YACs** back into the host of origin. Early successes suggest that such tools will provide a powerful approach to the structural and functional analyses of genes and the genetic manipulation of higher eukaryotic genomes. There is little doubt that these procedures will contribute greatly to the realization of the ultimate goal of genome anal.: a detailed understanding of the genetics and biol. of complex organisms.

L7 ANSWER 40 OF 52 COPYRIGHT 1993 ACS
AN CA115(9):86708q
TI Generation of a nested series of interstitial deletions in yeast

artificial chromosomes carrying human DNA
SO Proc. Natl. Acad. Sci. U. S. A., 88(13), 5744-8
AU Campbell, Colin; Gulati, Rajiv; Nandi, Asit K.; Floy, Kimberly; Hieter, Philip; Kucherlapati, Raju S.
PY 1991
AN CA115(9):86708q
AB A nested series of interstitial deletions were generated in a fragment of human X chromosome-derived DNA cloned into a **yeast artificial chromosome** (**YAC**) vector. A yeast strain carrying the **YAC** was transformed with a linear **recombination** substrate contg. at one end a sequence that is uniquely represented on the **YAC** and at the other end a truncated long interspersed repetitive element (LINE 1, or L1). **Homologous recombination** between the **YAC** and the input DNA resulted in a nested series of interstitial deletions, the largest of which was 500 kilobases. In combination with terminal deletions that can be generated through **homologous recombination**, the interstitial deletions are useful for mapping and studying gene structure-function relationships.

L7 ANSWER 41 OF 52 COPYRIGHT 1993 ACS
AN CA114(19):179667v
TI Stable integration and expression in mouse cells of yeast artificial chromosomes harboring human genes
SO Proc. Natl. Acad. Sci. U. S. A., 88(6), 2179-83
AU Eliceiri, Brian; Labella, Tullio; Hagino, Yoshi; Srivastava, Anand; Schlessinger, David; Pilia, Giuseppe; Palmieri, Giuseppe; D'Urso, Michele
PY 1991
AN CA114(19):179667v
AB The authors developed a way to fit **yeast artificial chromosomes** (**YACs**) with markers that permit the selection of stably transformed mammalian cells, and detd. the fate and expression of such **YACs** contg. the genes for human rRNA (rDNA) or glucose-6-phosphate dehydrogenase (G6PD). The **YACs** in the yeast cell are retrofitted with selectable markers by **homologous recombination** with the URA3 gene of one vector arm. The DNA fragment introduced contains a LYS2 marker selective in yeast and a thymidine kinase (TK) marker selective in TK-deficient cells, bracketed by portions of the URA3 sequence that disrupt the endogenous gene during the **recombination** event. Analyses of transformed L-M TK- mouse cells showed that **YACs** contg. rDNA or G6PD were incorporated in essentially intact form into the mammalian cell DNA. For G6PD, a single copy of the transfected **YAC** was found in each of 2 transformants analyzed and was fully expressed, producing the expected human isoenzyme as well as the heterodimer composed of the human gene product and the endogenous mouse gene product.

L7 ANSWER 42 OF 52 COPYRIGHT 1993 ACS
AN CA114(19):179562g
TI Detection of **homologous recombination** between **yeast artificial chromosomes** with overlapping inserts
SO Nucleic Acids Res., 19(5), 997-1000

AU Cellini, Alessandra; Lacatena, Rosa M.; Tocchini-Valentini, Glauco P.
PY 1991
AN CA114(19):179562g
AB A system is developed which facilitates the detection of **recombination** between **yeast artificial chromosomes** (**YAC**'s) carrying **homologous** inserts. The system consists of a classical **YAC** vector, a new **YAC** vector and two appropriately labeled yeast strains of opposite mating type. The new **YAC** vector differs in markers from the canonical **YAC** vector. To test whether **homologous recombination** takes place, phage lambda DNA was cloned in the two vectors to provide a region of homol. The two constructs were then introduced into yeast strains of opposite mating type in which the endogenous genes for the selective markers present in the vectors are not expressed. Artificial chromosomes obtained by meiotic **recombination** are detected in the spores resulting from the mating.

L7 ANSWER 43 OF 52 COPYRIGHT 1993 ACS
AN CA114(11):95870t
TI Modification and manipulation of mammalian DNA cloned as YACs
SO Genet. Anal.: Tech. Appl., 7(5), 107-13
AU Reeves, Roger H.; Pavan, William J.; Hieter, Philip
PY 1990
AN CA114(11):95870t
AB A review with 12 refs. Fragmentation and integration systems are now in place which permit manipulation of **YAC**-cloned segments in ways analogous to those available for sequences cloned by conventional procedures. **YAC** cloning offers several distinct advantages, beginning with the size of segments included in **YACs**. Ease of chromosome walking is related directly to the size of the steps. **YACs** readily increase this distance by more than an order of magnitude. For studies of gene regulation, the incorporation of large amts. of surrounding sequences guarantees the inclusion of all cis- elements, and may include sufficient information to confer higher orders of regulation, e.g., affecting chromatin conformation in the vicinity of the gene. **Homologous recombination**-based modification and screening systems available in yeast offer unique possibilities for identification and characterization of genes cloned in **YACs** . The development of procedures for moving **YACs** into cells and ultimately into the mammalian germ line completes the basic tools necessary to realize the tremendous potential of this system.

L7 ANSWER 44 OF 52 COPYRIGHT 1993 ACS
AN CA114(9):76353k
TI Site-directed, recombination-mediated mutagenesis of a complex gene locus
SO Nucleic Acids Res., 18(24), 7349-55
AU Barton, Michelle Craig; Hoekstra, Merl F.; Emerson, Beverly M.
PY 1990
AN CA114(9):76353k
AB A site-specific 17 bp insertion was constructed within a 38 kb chick globin gene cluster by employing the **recombination** abilities of Saccharomyces cerevisiae. This gene cluster contains 4 .beta.-type globin genes which share a high degree of sequence

homol. In this procedure, a small fragment of .beta.A-globin DNA contg. a 17 bp insertion is subcloned into a URA3-based yeast integrating vector (YIp). This mutated globin subclone is introduced into cells that carry the 38 kb globin cluster clone on a single-copy, circular vector derived from a **yeast artificial chromosome** (**YAC**). Insertion of the 17 bp oligomer is achieved by targeted integration of the YIp subclone. The **recombinant** contains the normal .beta.A-globin gene, the mutant gene and YIp vector sequences between the 2 copies. Excision of the vector sequences and one copy of the duplicated globin sequences by **homologous recombination** is required for cell survival when exposed to the selective agent 5-fluoroorotic acid, which is toxic to ura+ yeast cells. Depending upon the point of the cross-over, a ura- yeast cell bearing either a wild-type globin gene or a 17 bp insertion mutation will result. By restriction mapping and in vitro transcription anal., the .beta.A-globin gene contg. the 17 bp insert has no nonspecific mutations generated during the **recombination** and selection procedures. Specific mutations of regulatory regions, including protein-DNA binding sites, can be accurately targeted within extensive DNA clones by this method.

L7 ANSWER 45 OF 52 COPYRIGHT 1993 ACS
AN CA113(13):110427d
TI Modification and transfer into an embryonal carcinoma cell line of a 360-kilobase human-derived yeast artificial chromosome
SO Mol. Cell. Biol., 10(8), 4163-9
AU Pavan, William J.; Hieter, Philip; Reeves, Roger H.
PY 1990
AN CA113(13):110427d
AB A neomycin resistance cassette was integrated into the human-derived insert of a 360-kb **yeast artificial chromosome** (**YAC**) by targeting **homologous recombination** to Alu repeat sequences. The modified **YAC** was transferred to an embryonal carcinoma cell line (Bls-2) by using polyethylene glycol-mediated spheroplast fusion. A single copy of the human sequence was introduced intact and stably maintained in the absence of selection for over 40 generations. A substantial portion of the yeast genome was retained in hybrids in addn. to the **YAC**. Hybrid cells contg. the **YAC** retained the ability to differentiate when treated with retinoic acid. This approach provides a powerful tool for in vitro anal. because it can be used to modify any human DNA cloned as a **YAC** and to transfer large fragments of DNA intact into cultured mammalian cells, thereby facilitating functional studies of genes in the context of extensive flanking DNA sequences.

L7 ANSWER 46 OF 52 COPYRIGHT 1993 ACS
AN CA113(11):92753a
TI Isolation of cDNA clones using yeast artificial chromosome probes
SO Nucleic Acids Res., 18(13), 3913-17
AU Elvin, P.; Slynn, G.; Black, D.; Graham, A.; Butler, R.; Riley, J.; Anand, R.; Markham, A. F.
PY 1990
AN CA113(11):92753a
AB The cloning of large DNA fragments of hundreds of kilobases in yeast artificial chromosomes has simplified the anal. of regions of the

genome previously cloned by cosmid walking. The mapping of expressed sequences within cosmid contigs has relied on the assocn. of genes with sequence motifs defined by rare-cutting endonucleases, and the identification of sequence conservation between species. It was reasoned that if the contribution of repetitive sequences to filter hybridizations could be minimized, then the use of large cloned DNAs as hybridization probes to screen cDNA libraries would greatly simplify the characterization of hitherto unidentified genes. The use of this approach is demonstrated by using a YAC, contg. 180kb of human genomic DNA including the aldose reductase gene, as a probe to isolate an aldose reductase cDNA from a .lambda.gt11 human fetal liver cDNA library.

L7 ANSWER 47 OF 52 COPYRIGHT 1993 ACS
AN CA112(25):230816f
TI Isolation of single-copy-sequence clones from a yeast artificial chromosome library of randomly-sheared Arabidopsis thaliana DNA
SO Plant Mol. Biol., 14(4), 561-8
AU Ward, Eric R.; Jen, George C.
PY 1990
AN CA112(25):230816f
AB The construction of a **yeast artificial chromosome** (**YAC**) library from the A. thaliana genome is described. Randomly sheared high mol. wt. source DNA was extd. from frozen, ground leaf tissue and blunt-end-ligated to the vector pYAC3. By size-fractionating the ligation products, an av. clone size of 150 kb was achieved. Approx. 6% of the **YACs** contained inserts from the chloroplast genome. Clones equiv. to greater than 4 A. thaliana haploid nuclear genomes were screened. **YACs homologous** to 5 single-copy-sequence probes were isolated. The library should be useful for chromosome walking and genome mapping expts. In addn., the approach used for its construction should be applicable to other higher plant species.

L7 ANSWER 48 OF 52 COPYRIGHT 1993 ACS
AN CA112(17):153018t
TI Generation of deletion derivatives by targeted transformation of human-derived yeast artificial chromosomes
SO Proc. Natl. Acad. Sci. U. S. A., 87(4), 1300-4
AU Pavan, William J.; Hieter, Philip; Reeves, Roger H.
PY 1990
AN CA112(17):153018t
AB Mammalian DNA segments cloned as **yeast artificial chromosomes** (**YACs**) can be manipulated by DNA-mediated transformation when placed in an appropriate yeast genetic background. A fragmenting vector has been developed that can introduce a yeast telomere and selectable marker into human-derived **YACs** at specific sites by means of **homologous recombination**, deleting all sequences distal to the **recombination** site. A powerful application of the method uses a human Alu family repeat sequence to target **recombination** to multiple independent sites on a human-derived **YAC**. Sets of deletion derivs. generated by this procedure greatly facilitate restriction mapping of large genomic segments. Targeting **recombination** with single copy sequences, such as cDNAs, will have many addnl. applications. This approach establishes a paradigm for manipulation and

characterization of mammalian DNA segments cloned as **YACs**.

L7 ANSWER 49 OF 52 COPYRIGHT 1993 ACS
AN CA105(17):151229u
TI Interferon and butyrate treatment leads to a decreased sensitivity of NK target cells to lysis by **homologous** but not by heterologous effector cells
SO Nat. Immun. Cell Growth Regul., 5(4), 211-20
AU Laskay, Tamas; Kiessling, Rolf
PY 1986
AN CA105(17):151229u
AB Human K-562 and HHMS cells were pretreated with human **recombinant** interferon (IFN)-.gamma. and used as targets in natural killer (NK) assays against human and murine effector cells. A protective effect against NK lysis was obsd. only in the **homologous** assay. In cold target inhibition expts. IFN-treatment of K-562 cells led to a decrease in their capacity to act as competitors in the **homologous** NK assay, leaving their inhibitory capacity unaltered in the heterologous assay. In accordance with results obsd. using human NK targets, murine **YAC**-1 cells treated with mouse **recombinant** IFN-.gamma. did not lose their susceptibility to human NK cells. However, they were markedly less susceptible to lysis mediated by murine effectors. Butyrate, another compd. causing decreased sensitivity of K-562 cells for human natural killing, also failed to reduce the susceptibility against murine NK cells. Thus, the NK-resistant tumor target phenotype caused by IFN or differentiation-inducing agents can only be detected by **homologous** but not by heterologous effector cells. This suggests that major differences exist between the inter- and intraspecies NK killing mechanisms.

L7 ANSWER 50 OF 52 COPYRIGHT 1993 ACS
AN CA100(21):169274m
TI Chromosome assignment of two cloned DNA probes hybridizing predominantly to human sex chromosomes
SO Hum. Genet., 65(3), 257-61
AU Rappold, Gudrun A.; Cremer, T.; Cremer, C.; Back, W.; Bogenberger, J.; Cooke, H. J.
PY 1984
AN CA100(21):169274m
AB In situ hybridization expts. were carried out with 2 clones, **YACG** 35 and 2.8, which had been selected from 2 genomic libraries strongly enriched for the human Y chromosome. Besides the human Y chromosome, both sequences strongly hybridized to the human X chromosome, with few minor binding sites on autosomes. On the X chromosome, DNA from clone **YACG** 35 hybridized to the centromeric region and the distal part of the short arm (Xp2.2). On the Y chromosome, the sequence was assigned to 1 site situated in the border region between Yq1.1 and Yq1.2. DNA from clone 2.8 also hybridized to the centromeric region of the X and the distal part of the short arm (Xp2.2). On the Y, 2 binding sites were obsd. (Yp1.1 and Yq1.2). Thus, sex chromosomal sequences may be localized in **homologous** regions (as suggested from meiotic pairing) but also at ectopic sites.

L7 ANSWER 51 OF 52 COPYRIGHT 1993 ACS

AN CA98(3):15324f
TI NK cell specificity and possible target structures
SO Dev. Immunol., 17(Curr. Concepts Hum. Immunol. Cancer Immunomodulation), 283-90
AU Axberg, Inger; Nose, Masato; Wigzell, Hans
PY 1982
AN CA98(3):15324f
AB Treatment of the human cell lines MOLT-4 and K562 and the mouse cell line **YAC**-1 with trypsin reduced their sensitivity to natural killer (NK) lymphocyte-mediated cytolysis when tested with **homologous** effector cells. When tested with heterologous effector cells, the MOLT-4 and **YAC**-1 cell lines displayed no redn. in sensitivity. The mouse cell line L10A showed no redn. in sensitivity upon trypsin treatment when tested with either murine or human effector cells. Tunicamycin treatment of mouse myeloid leukemia cell line M1 with the glycosylation inhibitor tunicamycin decreased the sensitivity of the target cells for mouse NK cells, but increased the sensitivity for human NK cells. Pretreatment of the tumor cells with irradn. or mitomycin C prior to tunicamycin addn. completely inhibited cell division, did not affect the ability of tunicamycin to inhibit glycosylation, and inhibited the ability of tunicamycin to alter NK cell sensitivity. Apparently, human and murine NK cells recognize different target mols. and protease-sensitive targets can be relevant to NK cell-mediated killing across species barriers.

L7 ANSWER 52 OF 52 COPYRIGHT 1993 ACS
AN CA96(25):215726j
TI Immunoglobulin heavy chain gene rearrangement and transcription in murine T cell hybrids and T lymphomas
SO Proc. Natl. Acad. Sci. U. S. A., 79(9), 3015-19
AU Zuniga, Martha C.; D'Eustachio, Peter; Ruddle, Nancy H.
PY 1982
AN CA96(25):215726j
AB The arrangement was studied of Ig heavy chain const. (CH) and joining (JH) region genes in murine T-cell hybrid lines and in T-lymphomas. CH genes derived from both parental cell types were present in all hybrids for which polymorphism in sequences flanking CH genes permitted one to distinguish parental CH genes. All T lymphomas and T cell hybrids retained the C.alpha. gene in germ-line configuration and all but one cell line had germ-line C.mu. genes. Novel DNA fragments reactive with JH probes were obsd. in 6 of 9 T-cell hybrids, as well as in 2 T-lymphomas, WEH17.1 and **YAC** -1, but not in the fusion parent, BW5147. No RNA **homologous** to C.gamma.2b, C.alpha., or .gamma. genes was detected in any of the T-cell lines. T-cell lines contained poly(A)+ RNA **homologous** to a C.mu. cDNA probe. More importantly, in several cell lines the C.mu. RNAs were assocd. with membrane-bound polyribosomes. Apparently, both JH rearrangements and C.mu. rearrangements and C.mu. RNA prodn. occur in at least some mature, antigen-specific T-cells. They may therefore, reflect events in normal T-cell development and function related to those involved in the generation of the T-receptor for antigen.